

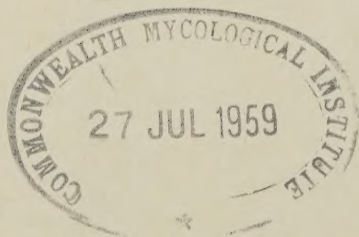
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EDITED BY

HENRY WELCH, Ph.D.

*and*

FELIX MARTI-IBÁÑEZ, M.D.

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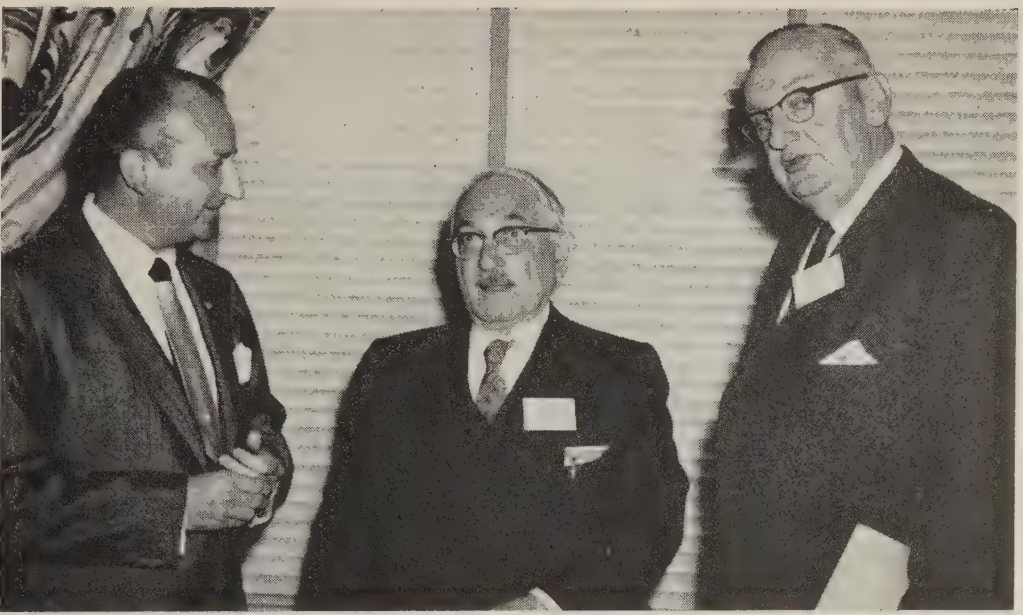
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Presidential greetings to the Antibiotics Symposium are read by (left to right) Dr. Henry Welch, Director, Division of Antibiotics, Food and Drug Administration, and Symposium Chairman; Sir Howard W. Florey, University of Oxford, Oxford, England; and Dr. Félix Martí-Ibáñez, Professor and Director of the Department of the History of Medicine, New York Medical College, Flower and Fifth Avenue Hospitals, who served as Moderator of the Historical Session.



View of audience at Sixth Annual Symposium on Antibiotics, during panel discussion on The Current Status of Erythromycin, Kanamycin, Novobiocin, Oleandomycin, Ristocetin, and Vancomycin, with Particular Reference to Their Use in Staphylococcal Disease, moderated by Dr. Maxwell Finland.



Dr. Selman A. Waksman (*center*), co-discoverer of streptomycin, is welcomed by Dr. Félix Martí-Ibáñez (*left*) and Dr. Henry Welch (*right*) at the opening of the Historical Session of the Symposium.



The first patient to receive Aureomycin ten years ago was Louise M. Ellis (*left*) treated for Rocky Mountain spotted fever. Three members of the Historical Panel, Sir Howard W. Florey (*third from left*), Dr. Selman A. Waksman (*fourth from left*), and Dr. Chester S. Keefer (*extreme right*) are also shown in the audience.

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DR HENRY WELCH, CHAIRMAN=  
SIXTH ANNUAL SYMPOSIUM ON ANTIBIOTICS  
CARE WILLARD HOTEL (BALLROOM)  
WASHDC=

PLEASE GIVE MY GREETINGS TO THOSE  
ATTENDING THE SIXTH ANNUAL SYMPOSIUM ON  
ANTIBIOTICS, AND A SPECIAL WELCOME TO YOUR  
DISTINGUISHED GUESTS FROM ABROAD.  
IN THE 30 YEARS SINCE THE DISCOVERY OF  
PENICILLIN, YOUR FIELD OF STUDY HAS  
GROWN ENORMOUSLY TO THE BENEFIT  
OF ALL.  
GATHERED HERE IN THE TRADITION OF FREE  
SCIENTIFIC EXCHANGE, YOUR SYMPOSIUM  
PROVIDES FRESH STIMULUS TO ADVANCE THE  
HEALTH OF THE WORLD COMMUNITY.  
BEST WISHES FOR A SPLENDID MEETING.=

DWIGHT D. EISENHOWER=

## Opening Remarks

HENRY WELCH

*Director, Division of Antibiotics, Food and Drug Administration,  
Department of Health, Education, and Welfare, Washington, D. C.*

Ladies and gentlemen, once again it is my pleasure to open another Symposium on Antibiotics, the Sixth in this series. I particularly want to greet our foreign guests who have traveled many thousands of miles to participate in this conference, which is concerned with subject matter that is worldwide in scope and of great importance to the health, welfare, and economy of all of us. On the program, there are 27 papers from 14 countries abroad and these will be presented by our colleagues from Argentina, Austria, Belgium, Canada, Czechoslovakia, England, France, Japan, Mexico, Peru, Scotland, Spain, Sweden, and Uruguay. From the United States 116 papers are to be presented by outstanding scientists in the field of antibiotics and chemotherapy.

Each year we on the program committee anticipate that fewer papers will be submitted but to date this has never happened. This year is no exception, since well over 200 abstracts were offered for presentation and it was most difficult for the committee to make their selections. We wish to say to those whose papers were not included that this may have been related to insufficient data in the abstract submitted on which to base a decision or that the subject matter had been thoroughly covered by others with more complete data. In any case, we are sorry that all could not be included on the program.

The tremendous quantities of antibiotics now produced (more than two and one-half million pounds yearly) in the United States alone has resulted in marked improvement in the treatment and prophylaxis of infectious diseases but along with these advances in therapy medical problems, some quite serious, have arisen. Many of these can and have been dealt with by proper and critical use of these drugs while others remain unsolved. One of the latter is the pressing problem of the resistant staphylococci and prevailing incidence of worldwide staphylococcal disease. Practically four sessions of the conference, including two panel discussions, will be devoted to the staphylococcal disease problem and the antistaphylococcal antibiotics.

Because of their importance, the program committee this year decided to include papers on chemotherapeutic agents other than antibiotics. A series of nine papers dealing with long-acting sulfonamides, nitrofurans, and quaternary compounds have been scheduled for Wednesday afternoon. Nearly an entire session has been assigned to the discussion of new antitumor agents. In addition, a variety of papers concerning sensitivity testing, phage typing, antibiotic inhibitors, and growth promotion are included for those interested in the in vitro and in vivo laboratory aspects of chemotherapy.

The first session of the Symposium, quite appropriately, is an historical one. It has been 30 years since the discovery of penicillin by Fleming and it has been 10 years since the broad-spectrum antibiotics became available for clinical use. We are most fortunate in having for our speakers some of the key people responsible for initiating the "antibiotic era" in this country; Sir Howard Florey who brought penicillin from England to us and stimulated the interest of our government and industry in its production; Dr. Selman Waksman in whose laboratory streptomycin,

the second major antibiotic, was discovered; Dr. Chester Keefer who organized and administered the first clinical programs on both penicillin and streptomycin, and Dr. Harry Dowling who is recognized internationally as an expert in the field of chemotherapy and particularly for his contributions in the field of broad-spectrum therapy.

Dr. Félix Martí-Ibáñez, medical historian, whose book *Men, Molds, and History*—a unique contribution to the history and development of the antibiotics—has just been published, will moderate the historical session. His address “The First Thirty Years” will relate his impression of the first three decades of antibiotic therapy.

*Editor in Chief of MD Medical Newsmagazine; Professor and  
Director of the Department of the History of Medicine,  
New York Medical College, Flower and Fifth Avenue Hospitals;  
Associate Editor of Antibiotics & Chemotherapy and of  
Antibiotic Medicine & Clinical Therapy, New York, N. Y.*

## THE MEANING OF THIS ANNIVERSARY

This Symposium coincides with the celebration of a memorable anniversary. An anniversary is a great occasion when it commemorates persons whose inventions are of immeasurable value in everyday life. Every time we switch on the electric light, pick up the telephone, or fly in a plane, we extend unconscious tribute to the persons whose genius brought comfort and efficiency into our lives, even though their names may have been lost in oblivion. But when it commemorates discoveries that save thousands of lives every day, the anniversary becomes a reverent tribute to those who, besides standard-bearers of civilization, were also benefactors to humanity. Such is this thirtieth anniversary of that historic moment when, in a modest laboratory in London's St. Mary's Hospital, a Scottish scientist observed the remarkable effect of a spore that had accidentally landed from the dull, smoky skies of the borough of Paddington upon a *Staphylococcus* culture plate.

Thirty years ago penicillin was discovered, and ten years ago broad-spectrum antibiotics were introduced. Let us note the difference between these two achievements of the Antibiotic Age. The first was accomplished by one man alone, as in the days of Pasteur; the second by a team of men dedicated to tracking down an antibiotic as persistently as safari hunters track down their quarry. This latter fact detracts nothing from the historical importance of the event, but it marks the development of a new mental and social attitude in research.

The Antibiotic Age is one more facet of our extraordinary epoch, which unquestionably deserves the appellation of "time-axis" applied by the philosopher Karl Jaspers to moments of portentous historical meaning, such as 500 B.C., when simultaneously in China, India, and Greece, fired by the words of Confucius, Buddha, and the Greek philosophers, human dignity and conscience were born and the great religions of the world began. Ours is another memorable "time-axis" because of the great discoveries made in fields of knowledge whose common-interest frontiers are as removed from one another as were in space the frontiers of the ancient cultures. So, in nuclear physics, cosmic astronautics, abstract art, depth psychology, and antibiotic research, revolutions have taken place leaving an indelible mark on the cultural face of the world of today.

Just as a mirror faithfully reflects the lines imprinted by the passing years on the human face, lines which if well earned add only to its beauty and dignity, so does this annual Symposium—the mirror of antibiotic medicine—faithfully reflect with every passing year the ever-increasing complex changes in this the most important branch of modern therapeutics.

Nothing can give a better idea of what this Symposium represents in the light of history than the use of that winged Pegasus, swifter than the fastest jet plane, our own imagination, to compare what will be said here with what might have been said at similar symposia on infectious diseases held, let us say, 3000, 300, and 30 years ago.

Three thousand years ago this symposium might have been a gathering of priests and magi in Babylonia, under the shadow of the majestic *ziggurats*, from whose summits watch was kept on the passage of the golden caravan of stars. Disease being then attributed to possession by devils or a punishment from the gods, prognosis would have been founded on auguries, divination, hepatoscopy, or horoscopes, and therapy on magico-mystic rituals or empiric remedies; and interchange of ideas would have consisted in formulating spells and praising the gods.

Three hundred years ago, in 1658, our Symposium might have taken place in Baroque Europe, in one of the academies at Bologna or Padua, which were then beginning to replace the medieval universities. The participants, clad in flowing velvet robes and starched ruffled collars, their fingers bejeweled with glittering rings, would have presented their *observationes* or biographic clinical histories, would have discussed their patients as people affected by a natural process caused not by demons but by miasmas, and would have formulated a prognosis and treatment based on empiric remedies and supplications almost as primitive as those of the symposium 3000 years ago.

Thirty years ago, in 1928, the Symposium would have been very similar in appearance to this one. In the intervening 270 years since the previous Symposium of 1658 practically all that we know today about the basic concepts of infection, immunity, and natural history of certain infections had already been learned. However pneumonia was still considered the "captain of the soldiers of Death," as Osler described it; tuberculosis and typhus were decimating the planet; malaria and plague were endemic in wide zones of the earth, and the memory of mortal pandemics, like the influenza epidemic after the First World War, still made us tremble.

This swift historical cavalcade brings us to our present symposium, the thirtieth anniversary of the Antibiotic Age. We speak with pride of the Antibiotic Age; yet, just as such technical miracles as the airplane and television no longer excite us, so the tiny tablets and ampoules imprisoning the God-created magic discovered by man in the minute world of molds no longer even stir our thought.

It is a fact that while artistic work is intimately bound up with its creator, thus leading us to speak of Beethoven's *Ninth Symphony*, Cervantes' *Don Quixote*, or da Vinci's *Mona Lisa*, scientific work is synonymous with anonymity. Though every time we use a drug we are offering mute tribute to its discoverers, still we administer antidiphtherial serum, anti-smallpox vaccine, or morphine without giving a thought to Roux, Jenner, or Sertürner. It is essential therefore that we celebrate anniversaries like the present, to recall the founders of medicine, to rescue not only their work but their names also from the dust of oblivion.

#### THE FORGOTTEN PRECURSOR: HOMAGE TO DUCHESNE

Now is the time, therefore, to pay due honor to the memory of one of Fleming's forerunners who anticipated the discovery of penicillin by 31 years. I refer to Ernest Augustin Clement Duchesne, a French army physician who died of tuberculosis at the age of 38 years, who in 1897 published his doctoral thesis on the antibacterial

FACULTÉ DE MÉDECINE ET DE PHARMACIE DE LYON

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CONTRIBUTION A L'ÉTUDE  
DE LA  
**CONCURRENCE VITALE**  
**CHEZ LES MICROORGANISMES**

Antagonisme entre les Moisissures et les Microbes

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**THESE**

PRÉSENTÉE

A LA FACULTÉ DE MÉDECINE ET DE PHARMACIE DE LYON

Et soutenue publiquement le 17 Décembre 1897

POUR OBTENIR LE GRADE DE DOCTEUR EN MÉDECINE

PAR

**Ernest DUCHESNE**

Né le 30 mai 1874, à Paris (Seine),

Elève de l'École du Service de Santé Militaire.



**LYON**

ALEXANDRE REY, IMPRIMEUR DE LA FACULTÉ DE MÉDECINE

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Décembre 1897

FIG. 1. This is title page for Ernest Duchesne's thesis entitled "Contribution to the Study of the Life Struggle among Microorganisms; Antagonism between Molds and Microbes."

I. Les moisissures (mucedinées) ne se développent pas, ou disparaissent, tout au moins, très rapidement dans l'eau, sous un certain volume, et cela pour les principales raisons suivantes : a) l'exagération même de l'humidité ; b) le mouvement de la masse liquide ; c) enfin et surtout le résultat de la concurrence vitale.

II. Il existe, en effet, un antagonisme très marqué et incontestable entre les moisissures et les bactéries qui ont été simultanément semées dans l'eau ou dans un liquide nutritif quelconque, et cet antagonisme tourne le plus souvent au profit des bactéries en ce qui concerne, tout tout au moins, les processus de vitalité et de végétalité.

III. Si les microbes l'emportent ainsi presque constamment sur les moisissures, dans la lutte pour la vie, c'est par suite d'une plus grande résistance vitale et surtout d'une pullulation infiniment plus rapide due, elle-même, au phénomène de la bipartition ou scissiparité. Mais il ne semble pas que les toxines microbiennes soient appelées à jouer un rôle actif dans cette lutte et dans ses résultats.

IV. Les Moisissures, cependant, peuvent parfois voir cette lutte tourner à leur profit lorsque le milieu de culture leur est, par sa réaction, plus nettement favorable qu'aux bactéries, qu'elles ne s'y trouvent pas absolument submergées et qu'elles sont enfin, initialement, en proportion vraiment très prépondérante.

V. Il semble, d'autre part, résulter de quelques-unes de nos expériences, malheureusement trop peu nombreuses et qu'il importerait de répéter à nouveau et de contrôler, que certaines moisissures (*Penicillium glaucum*), inoculées à un animal en même temps que des cultures très virulentes de quelques microbes pathogènes (*B. coli* et *B. typhosus* d'Eberth), sont capables d'atténuer dans de très notables proportions la virulence de ces cultures bactériennes.

VI. On peut donc espérer qu'en poursuivant l'étude des faits de concurrence biologique entre moisissures et microbes, étude seulement ébauchée par nous et à laquelle nous n'avons d'autre prétention que d'avoir apporté ici une très modeste contribution, on arrivera, peut-être, à la découverte d'autres faits directement utiles et applicables à l'hygiène prophylactique et à la thérapeutique.

LE PRÉSIDENT DU TRIBUNAL  
LEPINE

Par son dévouement  
LE TRIBUNAL  
O. CUMPRAT

Par son dévouement  
LE TRIBUNAL  
LEPINE

I. The molds (mucedines) do not develop, or at any rate disappear very rapidly in water below a certain volume, the principal reasons for which are the following: a) the increase in moisture itself; b) the movement in the liquid mass; c) finally, and especially, the result of the struggle for life.

II. There is, in fact, a very marked and undeniable antagonism between molds and bacteria which have been simultaneously set in water or any liquid culture medium, and more often than not such antagonism turns out to the benefit of the bacteria, at least as far as life and growth processes are concerned.

III. The reason the microbes practically always prevail over the molds in the struggle for life is their greater vital resistance and above all their infinitely speedier multiplication, itself due to the phenomenon of bipartition or scissiparity (fissiparity). It does not, however, appear that microbial toxins are called upon to play an active part in that struggle and its outcome.

IV. The molds may, however, sometimes see that struggle turn out to their advantage when the culture medium in its reaction is more clearly favorable to them than to the bacteria, if they are not absolutely immersed in it, and when in fact they are at the beginning in a really preponderating proportion.

V. Furthermore, it seems from some of our experiments, which unfortunately are too few in number and which ought to be repeated again and checked, that certain molds (*Penicillium glaucum*), when inoculated into an animal simultaneously with extremely virulent cultures of certain pathogenic microbes (*B. coli* and *Eberthella typhosa*), are able to attenuate the virulence of such bacterial cultures to a remarkable degree.

VI. It is to be hoped therefore that in pursuing the study of the facts of biological competition between molds and microbes—merely outlined by ourselves and to which we have no claim other than submitting here a very modest contribution—the discovery of further facts directly useful and applicable to prophylactic hygiene and therapy may be attained.

action of Hyphomycetes and demonstrated experimentally that cultures of *Penicillium glaucum* decreased the virulence of highly pathogenic microbes, such as *Bacillus coli* or *Eberthella typhosa*, in inoculated animals. On this anniversary let us present a tribute of admiration to the genius of this forgotten French scientist, who alone in an obscure laboratory anticipated the Antibiotic Age.

Nothing can enlighten us so well on how antibiotics might have changed the course of history than speculating on what they could have done for humanity both collectively and individually in the past.

Collectively, antibiotics might have stopped the great pandemics that changed the course of humanity—for instance, the 10 plagues of Egypt, the plague of Athens, Justinian's plague, the Black Death, the syphilis epidemics of the Renaissance, the Great Plague of London, yellow fever in the United States, typhoid in the Boer War, typhus in the Balkans, and the influenza pandemic of 1918-1919.

Individually, there are countless examples of what antibiotics might have prevented. Had Henry VIII's syphilis been treated with penicillin, his wives might have borne him an heir, instead of miscarrying every time, and would have been saved from the scaffold, while Henry himself not needing divorce might not have broken with the Pope nor supported the Protestant Church of England; Charles V might have been saved from syphilis and prolonged his reign, thus extending the might of the Spanish Empire; and had Lenin been cured of syphilis, he might never have reached the dizzy heights of general paresis from which he planned the Russian Revolution.

But all this belongs to the spectacular side of history. Here we are interested in appraising the changes undergone in these past 30 years by the *men*, the *research*, and the *results* of antibiotic medicine.

#### ANTIBIOTICS: THE WORK OF RESEARCH VETERANS

The first thing we observe on contemplating the discovery of antibiotics, is that practically every discoverer was a man ripe in years. Almost all discoveries in antibiotics have been made by men between the ages of 45 and 60. This fact must please us all—the young, because it confirms that scientific creation has no age limit; the mature, because it proves that they can still do as Don Quixote, who nearing his sixties set forth on his sorry nag to seek immortality. This maturity of antibiotic scientists is a symbolic herald of our Geriatric Era.

What does this mean? First of all, it means that in medicine as in history there are “young times” and “old times.” There are also branches, such as surgery, that will increasingly become fields of action for the young, just as there are branches, like psychiatry, where the main concepts were formulated by mature men. From the times of Pasteur, microbiology also has attracted men of maturity. Antibiotic medicine, the youngest of modern therapeutic branches, is the basic work of a team of illustrious mature veterans.

#### TEAMWORK AND INDUCED “LUCKY ACCIDENTS”

Antibiotic research has also changed during these 30 years. Originally there were innumerable lone investigators, but today the work is done in teams. The lone struggling scientist has been practically replaced by “commandos” in a “combined operation,” just as in the Baroque the “corporative” savant of the academies replaced the lone Renaissance scholar.

Similarly, discovery as a lucky accident has been replaced by discovery as the result of planning and organization. “Lucky accidents” were almost the rule in the history of research. Archimedes accidentally discovered a law of hydrostatics while investigating the adulteration of gold in the royal crown; Scribanus, physician to the

emperor Claudius, anticipated "electroshock" when he applied an electric eel to the forehead of a patient with migraine; by accident Roentgen discovered the rays now bearing his name; a milkmaid's casual remark led to Jenner's discovery of anti-smallpox vaccine; Pasteur's first great discovery occurred when through accidental contamination a fungus (penicillin!) developed in a solution containing the two isomers of tartaric acid; Ringer's solution, George Oliver's adrenalin, Sir Henry Dale's acetylcholine—all these were accidental discoveries. And a glorious accident befell Fleming, initiating the Antibiotic Age.

Today antibiotic research induces such accidents by experimentally producing the conditions leading to them. Likewise, we have stepped from experimental production in the laboratory to the development of gigantic research and isolation programs in the vast research departments of the pharmaceutical industry, which on another occasion I called the "scientific subconscious of a nation," where great discoveries in antibiotics are incubated.

#### LABORATORY AND MEDICINE

The laboratory has increasingly gained greater importance during these 30 years of the Antibiotic Age. In the Middle Ages Medicine was made by clerical physicians in monastery libraries lined with the splended tapestry of ivory- and gold-bound tomes and deep in centuries-old dust turned iridescent by the golden sun; in the last century Medicine was made in hospitals; in our time, it is made largely in laboratories, which takes the investigation of the sick one step beyond what our own senses might reveal.

#### ANTIBIOTICS: FROM "HEROIC" SPECIFIC THERAPY TO UNIVERSAL MEDICATION

Antibiotic therapy is the outcome of a long historical evolution that started about 6000 years ago. Back in those days, man treated disease with spells, herbs, and metals to drive out the causal agent, which was considered to be a demon, miasma, or germ; he then passed to the use of sera and vaccines to reinforce the body's natural defenses; aimed "magic bullets" at the bacteria; arrested their development by "putting them to sleep" with sulfonamides, enabling the leukocytes to sweep them away with their magic broom; tried to interfere with the bacteria's metabolism by throwing a monkey wrench into their biological machinery; fenced in the bacteria with the "magic wall" of penicillin; and finally he employed microbial antagonisms for therapeutic purposes.

In 30 years antibiotics have progressed from a "heroic" specific therapy to universal medication. This trend is the result of the conversion of antibiotics from an emergency medication to a routine medication which on occasions takes the place of precise diagnosis. The picture of infectious nosological entities has thus been altered, and contributing further to it has been the change in the natural history of a disease and in the biological cycle of its causal agent. Other features of interest have been the increasing synthesis of antibiotics in the laboratory independent of natural sources, and the improvement of their vehicles to assure greater tissue penetration, longer effectiveness, and reduced toxicity.

#### THE NEW PROBLEMS: RESISTANCE, METABOLIC ATTACK, BIOLOGICAL SYMBIOSIS AND NEW SOURCES OF ANTIBIOTICS

Antibiotic medicine has also encountered many problems. Of enormous impor-

tance is the growing resistance to antibiotics of certain microbes, as indicated by recent outbreaks of infections by antibiotic-resistant staphylococci in hospitals, which have been transformed into veritable culture foci on the living plate of the human being.

Another present-day problem is finding antibiotics that will attack the microbic forms of life still immune to them. Possibly the answer lies in discovering fresh forms of metabolic attack against such germs, in learning more about their biological cycles, or in developing a greater biological immunity in the human being. As Dubos said (thereby reviving one of Bernard Shaw's witticisms), probably the best thing would be not to destroy the germs but to learn to live with them in biological symbiosis, on a "live and let live" policy. We may also end up by reducing to the essential the drugs now being used, which recalls Osler's words: "The young physician starts life with twenty drugs for each disease, and the old physician ends life with one drug for twenty diseases."

It is also vital to develop fresh sources of antibiotics. The cure for many mortal infections may perhaps lie in the depths of the sea. Another tremendous source, despite its spatial smallness, may be the human body and its intercellular "inner sea," whose humors probably contain defensive powers as phenomenal as those of the waters of the high seas. Since Fleming investigated lysozyme, many ferments and hormones have been discovered, but a great many more remain to be discovered.

Finally, it is necessary to verify *how* and *where* the antibiotics work, and how much of their action is exerted on the microbe and how much on the surrounding cells and humors. We have to investigate the action level of antibiotics in the body, a level that may lie in the deep planes of tissular metabolism or in the circulating humors, and also whether antibiotics create a system of chain reactions involving the antibiotic, the microbe, and the humoral medium, that "internal sea" where the microbe, cell, and antibiotic lie submerged.

#### TOWARD A PROPHYLACTIC ANTIBIOTIC THERAPY

In the medicine of tomorrow the role of antibiotics will become increasingly more prophylactic as the interval between the discovery of a drug and its clinical application becomes shorter and shorter. For a long time mankind was deprived of the benefits of many drugs simply because it failed to use them until long after their discovery. But we have learned the lesson and are now reverting to the times of Paracelsus, when a drug was put into use as soon as it was discovered. On the other hand, in modern times, the sulfonamides, discovered in 1908, were not employed until 27 years later; isoniazid, discovered in 1912, was not applied until 40 years later, and penicillin, discovered in 1928, was not used until 13 years later. These delays were often caused by fear of toxicity. It is now high time to convince ourselves that a drug's toxicity often runs parallel with its therapeutic activity, demanding not its rejection but merely greater precision in its use.

#### THE NEWER PHILOSOPHICAL CONCEPTS OF DISEASE

The revision of our concept of the nature of disease is of increasing importance for the future application of antibiotics.

Today we agree that man is not only Nature but History. Man is what he does, the succession of moments in his life, his passage as a spatial form through time, a form always subject to the forces of his genetic equation, his environment, his

internal stresses, and his free will, all of which create his biography, of which disease is a part. Accepting clinical history as pathography or *graphia* of the *pathos*, we also accept the concept of the patient as a *whole*. The human being is regarded as a somatopsychic unit in motion, "making itself in the course of time." Disease therefore is disharmonious living, an abnormal and painful way of life, and infections are something more than a simple reaction among microorganisms, antibodies, and phagocytes. Disease is a series of interactions between an etiological cause and the patient, and infection itself is "a germ-time sequence crossing the space-time sequence."

Antibiotic therapy must take these concepts into account if besides fighting the causal microbe it also strives to fortify the patient's "whole person." According to Henri Laborit, it is more important to reduce the organism's total response to the morbid attack than the morbid attack itself. The drugs of tomorrow may be used to lessen excessive organic response to microbial attack, just as those of today are used to destroy the attacking stimulus.

#### WORDS AND SCIENCE

On this thirtieth anniversary of the discovery of antibiotics we can do no less than acknowledge the supreme lesson in humility that antibiotics have taught us, for these, the most important drugs of our times, originally come from the humble, diminutive world of molds and bacteria, thus bearing witness to the importance of the minute in this vast world of ours.

Antibiotics have also afforded us the unique pleasure of inventing new scientific concepts. Of the pleasures the scientist may have in his work, none is more satisfying than that of formulating a new concept. It is as delectable as the addition of a fresh batch of beauties to his harem was to Harun-al-Rashid in *The Thousand and One Nights*. Science also has its thousand and one nights, in which the supreme enjoyment is to feel, as Ortega y Gasset once pointed out, what the Greeks must have felt when they discovered scientific thought and discussed it in the common tongue.

Using metaphor (science's greatest semantic instrument), the Greek philosopher-physicians observed that by dressing a scientific concept in worn out common words the latter sparkled anew, as though a brilliant gem had suddenly been pinned on their threadbare semantic form. In its new sense, the common word ceased to be a workworn nag, exhausted from hard and constant use, and was transfigured into a winged Pegasus of philosophic thought. Similarly, in antibiotic medicine, we have been drawing from the bottomless sack of popular knowledge modest words which, kindled by the gem-like flame of a scientific idea, have been transformed into bright new technical terms.

However, there is one word that has not varied through the centuries and to which the ages have done nothing except add carats to its value. The word that on Hippocrates' lips meant love and kindness; on Galen's, experiment and curiosity; on Vesalius', a craving for wisdom and a passion for scientific honesty; on Harvey's, devotion and scientific ingenuity; on Pasteur's, industry and gentility; and goodness and genius on Fleming's. The word to which these thirty years of labor on antibiotics and more than six thousand years of endeavor and success in the art of healing pay tribute. The word synonymous with goodness and wisdom and the craving for service and abnegation by man for man. The word, MEDICINE.

## Introduction of Doctor Howard W. Florey

DR. MARTI-IBAÑEZ (Moderator). Never before in these symposia have we felt the presence of History as we do at this particular session. There are two kinds of history: the past history recorded in books, and the living history that we ourselves are making. It is this living history, wrought by the glowing thought of investigators, the equanimous activity of the clinician, and the wise word of the educator, that we so strongly feel today in this auditorium.

The classic custom is for the moderator to introduce the speakers to the audience. I am going to reverse that ritual. In deference to our illustrious visitors, I am going to introduce the *audience* to the speakers.

This audience, which unfailingly for six years has attended this Symposium, is composed of distinguished representatives of hospitals, laboratories, universities, and the pharmaceutical industry, and of veterans of medicine, chemistry, bacteriology, biochemistry, pharmacology, and allied sciences. Following the paths originally marked out by the pioneers of Antibiotic Medicine, or paths they themselves have cut out through the antibiotic jungle, all these men here today are with their toil writing the future history of antibiotic medicine.

Such is the audience to whom the speakers, who represent the aristocracy of scientific thought, are going to say what the Antibiotic Era means to them today, 30 years after its beginning. Their words have great value, not only because in your own youth you lived the youth of the Antibiotic Era, but because your thought, your word, and your work has helped our age to redeem itself from its scarlet balance-sheet of wars and revolutions.

I would like now to introduce our first speaker. The tradition of cooperation between British and American scientists dates back several centuries, to the times when some of the great figures in American medicine were educated in English and Scottish universities. In the case of antibiotic medicine, England has the glory of having discovered and first applied penicillin. In 1941, Dr. Howard Florey, who, with Chain and Heatley, introduced penicillin in clinical medicine, came to the United States seeking support for the production of penicillin on a large scale.

Seventeen years have passed. Sir Howard is here with us today. We hope that he will regard this meeting as his own scientific fireside, lending us of that voice, science, and conscience which, with Fleming and Chain, won him the Nobel Prize in 1945 and the reverent admiration of humanity for all time.

Dr. Florey's life is that of a "pure" scientist, meaning a scientist for whom *re-search* is preceded by *search*, not only for scientific ideals but also for the ultimate and burning truth. To have him among us is to relive a shining page in the history of Medicine through the man who himself wrote it. Since 1940, when thanks to him the physician's armament of mercy was reinforced with penicillin, his contributions to antibiotic medicine have been many and remarkable, placing him among the men who with their work shed a bright light upon the world of science. Possibly, however, his greatest hour was during the heroic years of the Second World War, when spanning the vast waters of the Atlantic, he laid a bridge of science and humanity between Great Britain and the United States. Over this bridge there now flows between the two countries the noble stream of that miracle of the human intelligence, Antibiotic Medicine. Ladies and Gentlemen, Sir Howard Florey, of the William Dunn School of Pathology of Oxford.

*Sir William Dunn School of Pathology,  
University of Oxford, Oxford, England*

First of all, I should like to thank you very much for having me at this Symposium on Antibiotics. In the letter inviting me to come, Dr. Welch said that it had been decided to have an historical session on the first morning. He put the word "historical" in quotation marks. I do not know whether that indicated that he takes a cynical view of the efforts of historians, but, whether he does or not, the quotation marks remind one to be cautious in presenting anything purporting to be historical. It usually appears to be fairly easy to ascertain most of the bare facts relating to observations and experiments, but it is quite another matter to put them in perspective and to make a coherent and chronologically satisfying and, may one say, true picture of the march of events.

F. L. Lucas,<sup>1</sup> in discussing some of the pitfalls of writing history, says, "There is a well-known story that Sir Walter Raleigh once witnessed from his prison window in the Tower some brawl in the courtyard below; then, discussing it later with a friend who had watched it likewise, was so staggered by their contradictory impressions of what had happened under their own eyes, that he flung aside in despair his *History of the World*."

Lucas also tells a story about the Duke of Wellington: "Even Wellington," he says, "was goaded by the legends that quickly gathered round Waterloo, to exclaim 'I begin to believe I was not there myself,' " and Lucas quotes, I think possibly with some degree of approval, Henry Ford's verdict on history, which was expressed in "one curt, contemptuous monosyllable—'Bunk.' "

Let me then, an amateur historian, approach the analysis of historical matters with circumspection.

I have given the title "Penicillin in Perspective" to this paper, and I will endeavor to put before you what seem to me to be important ideas and experiments that have led to the understanding of antibiotics today, and I will try to estimate what influence penicillin has had on modern medicine.

Penicillin has collected round it many myths and distortions that are perpetuated because most people have neither the time, the energy, nor, perhaps, the inclination to follow up the steps leading to discoveries. They are content with a few isolated facts connected by what they think may have happened before and after.

## EARLY WORK ON ANTIBIOTICS

Where and when did ideas about antibiosis first appear? It is clear that primitive peoples who used fungi for dressing wounds had no rational views of what they were doing. They may sometimes have observed apparent benefit from such application, but we must be careful in making such assumptions. We should not forget that virtue was supposed to reside in cow dung and that medicinal properties were attributed to the excreta of the Dalai Lama—at least until he was rationalized recently.

Although the antagonistic action of one bacterium on another was described by Roberts, in 1874, and by Pasteur and Joubert, in 1877, it is in an article by Babès, written in 1885, that some modern ideas were first set down. Here is a translation

of a paragraph he wrote: "One of us has studied experimentally the way in which bacteria of a known species produce chemical substances or modify the culture medium in such a way as to harm bacteria of other species. If the study of the mutual antagonism of bacteria were sufficiently far advanced a disease caused by one bacterium could probably be treated by another. The reciprocal action exerted on each other by bacteria is much more obvious if one is sown after the other. The bacterium which is inoculated first on the gelatine acts in two ways on the one which is sown later (1) by its chemical action (2) by its vital function."

Thus was adumbrated in 1885, i.e., not so long after the recognition of the bacterial cause of some diseases, the view that microorganisms can produce chemical substances—what we know today as antibiotics—that interfere with the growth of other microorganisms.

From among a good deal of work on antibiosis conducted at the end of the nineteenth century and the beginning of the twentieth, I have time only to pick out a few particular points, which appear to me to be important.

#### PYOCYANASE

Firstly, let us consider pyocyanase, for it was used extensively in the clinic. Precisely what pyocyanase was is not now clear; it certainly was not the enzyme that Emmerich and Löw supposed it to be. It is likely to have been a mixture of the powerful antibacterial substances that *Pseudomonas pyocyanea* is now known to produce. At the beginning of this century it received extensive trials in the clinic, both parenterally and by local application. It was even injected intrathecally for the treatment of meningitis, with very alarming results, I may add. Its use by local application was probably fairly widespread, and it was manufactured commercially.

Not only was it used in human medicine, but it was used in animals for treating, among other things, mastitis in cows, much in the same way as penicillin is used today.

A fundamental point about the action of antibiotics was clearly grasped by Escherich who, in 1906, stated that the march of science had produced a substance that possessed high bactericidal capacity and, at the same time, did not harm tissues, as did previously known antiseptics. He was referring to pyocyanase. He clearly understood the importance of differential toxicity in antiseptics, as indeed did Lister.

The conception that chemical substances produced by microorganisms might act more powerfully against some microorganisms than others was also grasped, especially as a consequence of the work on pyocyanase.

#### LODE'S OBSERVATIONS

I find a paper by Lode in 1903 particularly interesting, for it has a modern air about it. He described investigations of an accidental contaminant, which he had found while preparing a plate of *Micrococcus tetragenus* for class demonstration. This organism, a gram-positive coccus, was shown to produce a diffusible substance that strongly inhibited anthrax bacilli and *Staphylococcus aureus* but not *Bacterium coli* or Friedländer's bacillus. Lode showed that the antibiotic fluid was bactericidal and that the active substance was not an enzyme, though it was thermolabile, being inactivated slowly by heat. It could be dried by vacuum distillation and was soluble in alcohol but not in ether. Furthermore, and this is an important point to note, he showed that the microorganism and its metabolic products were not toxic to animals.

He went further and showed that his antibioticly active substance had no chemotherapeutic effect on artificial infection in mice. Lode's work could, in fact, well be a description of some antibiotic investigation of the present day.

There are, of course, dozens of papers describing interesting observations on antibiosis at the end of the nineteenth century and the beginning of the twentieth, and it is quite clear that most of the fundamental ideas about antibiotics had been expressed some 50 to 75 years ago.

#### DISCOVERY OF PENICILLIN

Let us now come to consider the discovery of penicillin against the background of knowledge that I have just shortly sketched.

As I did not know Fleming well, I have made use of a biographical memoir,<sup>2</sup> published by the Royal Society and written by Leonard Colebrook who knew Fleming well. He describes Fleming's career as a bacteriologist, working under Almroth Wright, especially during the First World War. They investigated wound infection and the failure of antiseptics in its treatment. He wrote that Wright and Fleming concluded "that two factors were chiefly responsible for the failure: First, the antiseptics did not reach the microbes because these had very often been implanted deeply in the substance of bones, cartilage, muscle, etc. And, secondly, the antibacterial potency of the solutions used was very much, and very quickly, reduced by combination with albuminous and cellular elements in exuded lymph, pus, blood, and the fixed tissue in the wounds; and the solutions thus reduced in strength were sometimes actually harmful, because they destroyed the patient's leucocytes, which, if unharmed and given favourable conditions, constituted a very effective natural defence mechanism."

"The intellectual work which lay behind the formulation of these two important conclusions (and others) was almost entirely Wright's (he 'saw with his mind' where the truth must lie), but Fleming, who shared in the work, made valuable contributions to it—especially in the technical sphere."

Fleming was a master technician with the test and pipette, and his work with Wright using such methods "prepared his mind," to quote Colebrook again, "in some measure for the discovery in 1922, of the remarkable microbe-dissolving ferment in nasal secretions which he named 'lysozyme.'"

In 1928, he discovered another antibacterial agent, elaborated by a *Penicillium*. During observations on some staphylococcal colonies growing on an agar plate culture, he noticed that some of them, which were growing in the neighborhood of a contaminating fungus, became translucent. They were apparently being lysed. Fleming, being a very acute observer, had his attention arrested by this unusual occurrence. He grew the *Penicillium* on the surface of nutrient broth and found that an antibacterial substance was secreted into the medium. This broth he called "penicillin." He found that it was considerably more harmful to bacteria than to the white cells of the blood, a sharp contrast with other antiseptics with which he and Wright had previously worked. Indeed, he could dilute the broth 800 times, and the growth of some sensitive organisms was still interfered with. He made many observations on its bacteriological properties, defining the organisms against which it was active, finding that gram-positive organisms in general were affected, while the gram-negative ones were unaffected. He injected intravenously 20 ml. of the active broth into rabbits, and stated that it caused no more disturbance than the injection of a similar amount of broth on which nothing had grown. As he said later, "We

have been using it in the laboratory for over 10 years as a method of differential culture. It was used in a few cases as a local antiseptic, but although it gave reasonably good results the trouble of making it seemed not worth while."

I think Fleming was perfectly sincere when he said that he was not influenced in his work on penicillin by anything that had been written or discovered before about antibiosis. He, like most of us, was not deeply conversant with all the relevant literature. Nevertheless, no new scientific ideas were involved in the discovery of penicillin.

Nothing emerges from Colebrook's memoir<sup>2</sup> or from Fleming's own writings to suggest that he had in mind the idea that penicillin might be a systemic chemotherapeutic agent in the sense that it could enter the blood stream and so circulate to infected parts without harming the host.

We can speculate as to why he did not discover this chemotherapeutic property. It is relatively simple to calculate that there must have been a sufficient concentration of penicillin in the crude broth that Fleming had in his hands to have enabled him to show its curative properties on experimental streptococcal infections in mice. Such experimental streptococcal infections had been used by Morgenroth and Levy as long ago as 1911 and were subsequently used in the early 1930's by Domagk when he was making his discovery of the chemotherapeutic properties of prontosil. Perhaps the reason was that he was unfamiliar with animal experiments, as most of his work had been on bacteriological culture and on isolated cells, such as leukocytes.

Fleming, as he himself has said, was no chemist and he therefore made no serious attempt to isolate the active substance in his broth. However, in the early 1930's, a very able group of chemists headed by Raistrick, who were among the foremost workers on fungal products, started to examine the active material. After partially purifying the substance, they concluded that it was too labile to work with conveniently and put it to one side. Some years ago, I asked Dr. Raistrick why he had dropped his investigations of penicillin at the stage he did, and he informed me that at that time they were interested mainly in investigating the chemical structure of fungal products which crystallized easily.

#### WORK AT OXFORD

Why was work on penicillin undertaken at Oxford in 1939? Perhaps we can go back some way here. During investigations on intestinal mucous secretion carried out in Cambridge in the 1920's, I became acquainted with lysozyme. When I went to Sheffield, Mrs. Harrison started to examine the substrate on which the enzyme acted. When I went to Oxford, the work of investigating the action of lysozyme was taken up by Epstein, an American Rhodes Scholar, under Chain's direction. With this background of interest in antibacterial substances, Chain read extensively and came across in the literature some of the now well-known antibiotic-producing organisms. He proposed that we should undertake an investigation of their properties. The choice was narrowed down to three, of which one was penicillin. Though this had been called a labile substance and put on the shelf by Raistrick and his colleagues, Chain read in their account that solutions retained their activity for some months, and he therefore concluded that it must be possible to find conditions in which the substance could be satisfactorily extracted. After some preliminary experiments by Epstein, Heatley joined the work and grew *Penicillium notatum* on a salt mixture into which it secreted penicillin. He also devised the solvent transfer

process, still in use commercially, by carrying Raistrick, Clutterbuck, and Lovell's observations a stage further, and the cylinder plate method of assay, which is both simple and quick and has been used widely in subsequent work. He also defined the "Oxford unit" by means of which different impure preparations of penicillin could be compared. This was subsequently adopted with slight modification as the international unit. This of course greatly facilitated the formulation of dosage schedules.

Even before penicillin had been extracted from the culture fluid, we had some idea that what we at that time considered quite strong solutions might be relatively nontoxic. Fleming had pointed out that the yellow droplets that appear on the surface of the fungus in culture are more active than the culture fluid. Some of these droplets were collected with a Pasteur pipette, and, as I was unfamiliar at that time with intravenous injections into mice, my pharmacological colleague Professor Burn injected 0.2 ml. of the yellow fluid into a mouse without any apparent effect.

I will not now outline all the difficulties that were encountered during one of the worst periods of the war, in order to obtain sufficient partly purified penicillin for further tests. The difficulties were overcome to the extent that a few hundred milligrams of crude penicillin were produced, and it was possible not only to investigate some of its chemical and bacteriological properties but to test out its effect on animals. The crude material, which we now know to have contained about 1 per cent of penicillin, had remarkably little toxicity to the whole animals or to their constituent cells, and it was strongly antibacterial—in fact, more powerful than most antiseptics. It was rapidly excreted by the kidneys after absorption from subcutaneous injections, but it was very poorly absorbed from the alimentary tract. Hence it was clear that when the time came to use it on man it would, in all probability, have to be given parenterally and by frequently repeated injection.

Mouse protection tests of the type used by Domagk in the investigation of sulfonamides gave staggeringly good results. We did not have to employ a statistician to work out whether the substance was chemotherapeutic to artificially infected mice.

It is difficult to convey the sense of urgency that existed at that time, but there can be little doubt that emotional factors concerned with helping the war effort played an important part in forwarding the work, although it had been started actually before the war and without any reference to its possibilities in war medicine.

At that time, the early 1940's, great emphasis was laid on the possibility of discovering its structural formula and thence of synthesizing it, so avoiding the troublesome brewing and extraction from brew containing very little active substance. Those working in my department were in collaboration with the organic chemists under Sir Robert Robinson in the Dyson Perrins Laboratory, and we had a most valued collaborator in Dr. Dorothy Hodgkin (Crowfoot) who, in the end, established by her admirable roentgen-ray analysis the structure of penicillin.

It is, of course, well known that there was much collaboration between those working in the United States and in Great Britain on the chemical structure and properties of penicillin. This work has been embedded in two large volumes and is a monument to what may be considered a fairly successful effort at international collaboration, a collaboration carried out under the stimulus of war.

Perhaps I can now summarize briefly what I consider that we accomplished by our work in Oxford. Firstly, the fungus was grown successfully on a liquid medium on a sufficient scale to enable raw material to be produced. A process was devised for the extraction of penicillin from the liquid and its concentration and purification. Its pharmacological properties were worked out in sufficient detail to enable us to

go to the clinic knowing fairly precisely by what routes the material would have to be administered and, with a fair degree of confidence, that it would not be toxic. Moreover, the effective dose could be calculated on the basis of the fairly frequent injections which were known to be necessary.

Secondly, the range of organisms against which penicillin was active was extended and, most important, it was found to be active in the presence of body fluids, including pus, a property that distinguished it clearly from the known sulfonamides.

Thirdly, animal experiments clearly established that it was a systemic chemotherapeutic agent, the organisms tested being a hemolytic *Streptococcus*, a *Staphylococcus*, and *Clostridium septicum*. The latter was particularly interesting, in view of the possibility of gas gangrene in war wounds.

Fourthly, based on this work, with enormous labor, sufficient penicillin was accumulated to administer to man, and the first patients ever to receive penicillin parenterally were treated in various Oxford hospitals. Few though the patients so treated were, the results were sufficiently gratifying to make it imperative to pursue vigorously the work on penicillin. I think the results obtained in our first small series gave a clear indication of the methods by which the material could best be used on man.

Fifthly, considerable strides were made in the understanding of the chemical structure of penicillin. One of the main causes of the instability of penicillin, namely contamination with bacteria that produced an enzyme penicillinase, was discovered. Other causes for the instability were found, and this knowledge enabled the material for use on man to be properly prepared and conserved.

#### WORK IN THE UNITED STATES

Let me now mention the work done in the United States. It is conceivable that the preoccupation with the chemical structure and possibility of synthesis retarded the investigation of the biological manufacture of penicillin in England. Be that as it may, it became apparent, in 1941, that it was unlikely that the substance would be produced in England at that time in a sufficient quantity to be really useful. You will remember that this was a grave stage of the war, when there was nothing to spare in Britain, either in men or materials, from the direct war effort. It was then that Heatley and I went to the United States in order to see if we could arouse interest in the possible manufacture of what we considered a quite remarkable substance. It should not be assumed that everybody in America shared our enthusiasm at that time. I can remember very clearly how a good friend of mine considered it quite impossible to do anything with a drug that took four barrels of beer, as he said, to treat one patient, nor, quite understandably, were all the pharmaceutical firms that we saw very receptive. Nevertheless, there was enough good will among some of those we visited for the matter to be taken further.

I will briefly summarize what I consider the three important steps that we owe to work in the United States. Firstly, there was deep fermentation. In the course of our first interview with Dr. Coghill and Dr. May at the Northern Regional Research Laboratory, Peoria, the possibility of deep fermentation was raised. Some weeks later, the first runs were set up in the experimental drums used for citric acid fermentation, and small but definite amounts of penicillin were formed. I cannot now go into the steps by which surface fermentation of the fungus was replaced by deep fermentation on what might be called a gigantic scale, but the whole of that step was accomplished by the chemical engineering skill of those in the United States.

The second fundamental contribution was the production of strains of the fungus yielding far more penicillin than Fleming's original strain. These strains were discovered at first by looking for better natural strains and later by inducing artificial mutants by roentgen-rays, ultraviolet light, and so on.

The third contribution was the improvement of fermentation media, especially by the addition of corn-steep liquor.

These three contributions made in the United States solved what at one time looked like the almost impossible task of making substantial quantities of penicillin.

Let us salute the very skilled chemical engineers as well as biologists who elaborated the present-day processes. Let us, too, salute the skill and enterprise of the chemical engineers who perfected by far the most technically exacting fermentation ever to be undertaken commercially.

#### FURTHER CLINICAL WORK

While this work was proceeding in the United States, we accumulated in England enough material prepared from surface cultures to treat more patients. Some of this material was supplied by commercial firms. The successful treatment of serious cases of infection, such as cavernous sinus thrombosis, and the knowledge gained about dosage encouraged trial on war wounds. To conserve the very small supplies, these were treated first by local application and, when more material became available, parenterally. This work established the fact that it was possible to sew up some severe wounds while bacteriostasis was maintained chemically—wounds that before would certainly have been left to granulate. This suturing of wounds not only produced quicker healing but far less scar tissue and consequent interference with function. I may say that it took a good deal to convince the older surgeons that suture could be done under such conditions, and I salute those surgeons who willingly tried the new suggestion. When it had been shown to be a safe method, I was told that in fact it had been done, *in suitable cases*, all along. Penicillin multiplied many fold the suitable cases.

During the early trials in North Africa, in which I had a modest hand largely as a "pep" talker, probably the first patient with gonorrhea ever to be treated was injected in a hospital at Sousse. He was an R.A.F. officer who had received some 250 Gm. of sulfonamides to no effect. The gonococcus was known to be an organism very sensitive to penicillin, and, although he could not strictly speaking be classed with the "war wounds," I was persuaded to let a little of the precious penicillin be used on him. I can remember the excitement as the gonococci disappeared within hours, and the immense satisfaction of curing the man in a couple of days.

I have traced the work done in Oxford through the laboratory to that in the clinic and to its use in war.

After industry in the United States had gotten well into production, relatively large quantities became available for clinical testing. Many more cases than we were able to treat in England were investigated. The results, when the material was properly used, were as favorable as those we had obtained. One new and very important observation was made, namely that spirochetes were sensitive to penicillin and that it was possible to treat syphilis with apparent success. With increasing supplies, doses tended to rise, because even enormous doses had no direct toxic effect and continuous improvements were made in the form of penicillin, making administration easier.

With the treatment of thousands of cases, the one serious drawback to penicillin

appeared, namely, allergic sensitization. Fortunately, development of resistance of organisms is rare, in spite of the superficial appearance to the contrary.

#### INFLUENCE OF PENICILLIN ON MEDICAL SCIENCE

I have already said that the discovery of penicillin by Fleming did not, in my view, add any new scientific ideas to our common stock. Did the discovery of the chemotherapeutic effects of penicillin and its further exploitation do more? I find it hard to say that they did. It is worth remembering that the work on penicillin from 1939 onward was done against the background of Domagk's recent discovery that prontosil could be administered systemically and that it showed a differential toxicity toward mammalian cells and bacteria. This in turn could be regarded as a realization, in the case of bacteria, of the concept of a systemic chemotherapeutic agent formulated by Ehrlich at the beginning of this century and fulfilled, for spirochetal infections, by salvarsan. That penicillin had a vastly greater therapeutic index than salvarsan or the sulfonamides does not imply that any new principles had been discovered.

There can, however, be little doubt that the remarkable properties of penicillin were a great stimulus in the hitherto slumbering field of antibiotic research, and that the results, as far as practical medicine is concerned, were extremely gratifying. One should, however, never forget the extraordinary good fortune that attended the work on penicillin. Dubos, you will recollect, had studied tyrothricin in 1939, before the work on penicillin was begun. He simply had the ill fortune to be working on a toxic compound, in much the same way as Emmerich and Löw had with pyocyanase.

I think, in estimating the influence on medical practice, one has to consider penicillin and other antibiotics in conjunction with the sulfonamides. The sulfonamides, by 1940, had already greatly influenced medical practice with regard to certain infectious diseases and brought about the situation in which those practicing medicine, if they were to do it with intelligence, had to have a considerable knowledge of bacteria. It was no longer sufficient to know that the *Streptococcus* was a chain of little round objects; a great deal more had to be known about its properties and especially about its reactions to the newer drugs. Today we can see the necessity for bacteriological study most clearly in hospitals, which are now realized to be, bacteriologically, very dirty places. Twenty years ago such ideas were not so clearly perceived.

#### LONG-TERM EFFECTS

If we look a little further, we can see that, possibly, the introduction of antibacterial drugs and of other means by which the death rate has been reduced may not be an unmixed blessing. In highly developed countries at the present time the average length of life is steadily increasing, but this means that active members of communities now have to carry a substantial burden of aging people. This burden is likely to become greater with the passage of time, and much thought is being given and more will be required to deal with this in the future. Another point of great importance is associated with the extraordinarily rapid increase in populations, particularly in Asia, that is occurring at the present time. This subject has been mentioned frequently enough; I merely bring it forward now to complete the picture of the possible unfavorable social consequences that can follow in the wake of medical progress.

I have endeavored to put before you some of what I consider to have been the essential steps in the development of penicillin. I could of course have told you a great deal more and kept you here several hours while doing so, but there are documents setting out these matters in greater detail for anyone interested.<sup>3</sup>

Perhaps I might end on a personal note. I have been extraordinarily well treated by my scientific colleagues, who have exaggerated the importance of the part I played in the development of penicillin and, indeed, have greatly overestimated my worth as a scientist. I am very grateful to them for having done so, but I would like to place on record, as I have done many times before, that the work on penicillin at Oxford could not have been accomplished without the working together of a number of people, including those in my laboratory. If I have not mentioned them all by name, it is because I have done so elsewhere and it would take up too much time today.

I have had a great extension of my experiences as the result of penicillin. I have seen much of the world and have many friends. There is only one serious regret that I have about the whole affair. That is, that I did not, on behalf of my colleagues and the laboratory, patent the processes by which penicillin was extracted. This, looking back on it, was a cardinal error, but at that time the patenting of medicinal substances by medically qualified people was heavily frowned upon both in Great Britain and the United States. However, if the processes of extracting penicillin had been patented it would have saved me a great deal of worry in subsequent years, for it seems to me that only by having available funds obtained by this means is it possible in Great Britain, at the present time, to be sure of keeping tried research workers and providing with certainty the income, security, and facilities which first-rate people in their thirties are surely entitled to. My department has, of course, been generously helped from many sources, for example by Lord Nuffield, the Albert and Mary Lasker Foundation, the Rockefeller Foundation, and drug firms of the United States and Great Britain. Nevertheless, I now find it almost impossible to find stable jobs for admirable young research workers in my own laboratory. They are the next generation, and I must confess that I feel rather frustrated in not being able to run a department so as to give positions and security to workers of promise.

May I conclude by thanking you most sincerely for listening to me so patiently and for having invited me to be present today.

## BIBLIOGRAPHY

1. LUCAS, F. L.: *The Search for Good Sense*, London, Cassell & Co., Ltd., 1958.
2. COLEBROOK, L.: Alexander Fleming, 1881–1955. *Biographical Memoirs of Fellows of The Royal Society* 2:117, 1956.
3. FLOREY, H. W.; CHAIN, E.; HEATLEY, N. G.; JENNINGS, M. A.; SANDERS, A. G.; ABRAHAM, E. P., AND FLOREY, M. E.: *Antibiotics*, New York and London, Oxford University Press, 1949, vol. 1, chap. 1; vol. 2, chap. 15.

## Introduction of Doctor Selman Waksman

DR. MARTI-IBAÑEZ (Moderator). Thank you, Sir Howard, for your splendid address, which has enabled us to contemplate the panorama of penicillin from the vantage post of the thought of one of the great pioneers in the antibiotic saga.

Medicine, like life itself, begins and ends in Philosophy. Philosophic thought led from the theoretic conceptions of the struggle for life in the invisible jungle of molds and bacteria to the laboratory where the scientist tamed these organisms and wrested from them their metabolic secrets.

With pride, we now introduce Dr. Selman Waksman, a philosopher of antibiotic research, whose work, as intellectually brilliant as it was poetic in conception, won him the 1945 Nobel Prize, the reverent admiration of the world, and the devout gratitude of legions of human beings who owe their lives to his philosophy and his discoveries.

Dr. Waksman brought to antibiotic research his philosophical determination to conquer Nature with her own laws. The result is well known to all of you and the whole world. But only he knows the long years of labor and struggle that preceded the moment when the flame that sheds the light of genius was kindled. Dr. Waksman, of the Institute of Microbiology, Rutgers, The State University.

SELMAN A. WAKSMAN

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This meeting is indeed an historic occasion. The growing knowledge of antibiotics in general and actinomycetes in particular was first announced to the world in these very halls, or at least within a short distance from here. Just a little more than 18 years ago, on April 23, 1940, I addressed the National Academy of Sciences in this city on the subject of "The Soil as a Source of Microorganisms Antagonistic to Disease-producing Bacteria."<sup>1</sup> In this address, I first reported on a culture of an *Actinomyces* isolated from the soil and its growth-inhibiting effect upon various bacteria. The active substance was soon isolated in crystalline form and designated, quite properly, actinomycin.

The first meetings of the Special Committee, appointed by the Committee on Medical Research of the Office of Scientific Research and Development for the study of the production of penicillin and other antimicrobial agents produced by microorganisms, were held here in Washington. The Committee on Chemotherapeutics of the National Research Council, acting together with the Committee on Medical Research, later replaced the previous Committee and supervised and coordinated much of the basic work done in this country on the antibiotics. I recall quite clearly the meeting held on June 14, 1945, at which recommendations concerning future policy for the study of streptomycin were examined. On May 31, 1946, this Committee reported on "Clinical Investigations of Streptomycin,"<sup>2</sup> and four weeks later (June 28, 1946), the first specifications for streptomycin standards were drawn up by the Food and Drug Administration.<sup>3</sup>

Late in 1946, the first series of regular lectures on antibiotics was held at the graduate school of the U.S.D.A. On January 31, 1947, the first symposium on antibiotics, following that held one year earlier at the New York Academy of Sciences,<sup>4</sup> was organized under the auspices of the U. S. Public Health Service. My address at that symposium dealt with the subject "Antibiotics of Actinomycetes, with Special Reference to Certain Challenging Problems, Notably the Development of Bacterial Resistance."<sup>5</sup> The first issue of the journal ANTIBIOTICS & CHEMOTHERAPY appeared on April 1, 1951. And finally, the first meeting of the Annual Symposium on Antibiotics was held here just five years ago.

It is thus logical that Washington should also be the place where the first historical conference devoted to the subject of antibiotics should be held.

## HISTORICAL BACKGROUND

No attempt will be made to review the historical background of antagonistic effects of actinomycetes upon bacteria and fungi and the production of antibiotic substances. Attention will only be called to certain observations and to a certain terminology that have underlain many of the developments in this field.

*Accidental Observations.* Gasperini<sup>6</sup> is credited with having been the first to report, in 1890, that certain actinomycetes are capable of bringing about the destruction of bacteria and fungi. Thirty years passed before Lieske<sup>7</sup> demonstrated, in 1921,

that not only are various actinomycetes capable of lysing cultures of different bacteria, but also that this action is selective in nature. Gratia and Dath,<sup>8</sup> in 1924, confirmed this observation and obtained, from the broth of a *Streptomyces* culture, a preparation that was designated as "mycolysate." This preparation was actually used in the treatment of a large number of clinical cases. Rosenthal<sup>9</sup> soon established that certain actinomycetes are capable of inhibiting the growth of diphtheria bacteria; this action was found to be due to the production of a thermostable active substance. Welsch<sup>10</sup> later designated as actinomycetin the preparation obtained from Gratia's *Streptomyces albus* culture, which was still later shown to consist of a bacteriolytic principle and of an enzyme;<sup>11</sup> dead gram-negative and living gram-positive bacteria were affected.

Several Russian investigators, notably Borodulina,<sup>12</sup> Nakhimovskaia,<sup>13</sup> and Krasilnikov and Koreniako,<sup>14</sup> reported on the antibacterial activities of various soil actinomycetes. Many of the cultures now known to belong to the genus *Streptomyces* were found capable of liberating an active thermostable substance into the medium. Kriss<sup>15</sup> attempted to isolate this substance from a strain of *Streptomyces violaceus* and came to the conclusion that it resembled lysozyme, although it differed considerably from the lysozyme preparation of Fleming.

While most of these observations were largely concerned with the antibacterial properties of actinomycetes, Alexopoulos and Herrick<sup>16</sup> demonstrated that as many as 38.8 per cent of these organisms were also effective against fungi.

*Results of Soil Studies.* Side by side with these observations of the antimicrobial activities of actinomycetes, systematic studies were being carried out by investigators interested in composts, microbial relationships in soil, and the problem of suppression of plant diseases. In 1917, Greig-Smith,<sup>17</sup> concerned with the growth of bacteria in the soil and the possible effect of toxic substances produced in the soil upon such growth, suggested that actinomycetes might be responsible for the formation of the toxic factor. The resistance of actinomycetes to adverse factors was observed by various students of soil microbes. In a systematic study of the effect of actinomycetes upon the decomposition of plant residues by bacteria and fungi, Waksman and Hutchings<sup>18</sup> demonstrated an antagonistic effect. Similar effects of saprophytic actinomycetes upon parasitic forms, notably those causing potato scab, were previously reported by Millard and Taylor,<sup>19</sup> McCormack,<sup>20</sup> Ken Knight,<sup>21</sup> Tims,<sup>22</sup> and various other investigators.

Thus, prior to 1940, there accumulated a body of knowledge on the actinomycetes of the soil and their capacity to exert a limiting effect upon the growth of various bacteria and fungi.

#### SCREENING PROGRAMS

In the search for actinomycetes capable of producing antibiotics, systematic screening programs were undertaken. It was soon recognized that the antibiotics of actinomycetes represent a great variety of compounds rather than a single chemical entity, as believed by the earlier investigators. The concept of a lysozyme-like material or a bacteriolytic substance soon gave way to the recognition of the fact that well-defined chemical systems are involved. Two important principles were established: (1) that these substances are each characterized by a specific antimicrobial spectrum; and (2) that each organism may produce more than one type of antibiotic substance.

The first screening surveys established that nearly 50 per cent of all the cultures isolated from soil or other natural substrates (mostly members of the genus *Streptomyces*) were active, largely against gram-positive bacteria. Much smaller numbers were also active against gram-negative bacteria. The antimicrobial activity of the organisms depended largely on the composition of the medium, conditions of cultivation, and methods of testing. These surveys were made in our laboratories and were later followed by those of Burkholder, Landerkin and Lockhead, Emerson, Routien and Finlay, and various other researchers. They also established the fact that the ability of an organism to produce a certain antibiotic is not a species but rather a strain or varietal characteristic. Thus, in the search for strains of *Streptomyces griseus* capable of producing streptomycin, in more than 100 cultures isolated from natural substrates only one was found capable of producing streptomycin; several produced other antibiotics, such as grisein, streptocin, and candicidin, but most produced no antibiotic at all. The ability of the same culture to form several antibiotics or the same antibiotic in several closely related or isomeric forms tended to complicate further the problems of isolation and identification of the antibiotics.

#### ISOLATION OF THE FIRST ANTIBIOTIC FROM CULTURES OF ACTINOMYCETES

Aside from actinomycetin and the lysozyme-like preparation, actinomycin deserves the credit for having been the first antibiotic isolated in a crystalline form from a culture of an actinomycete.<sup>23</sup> Closely related compounds were later isolated in numerous other laboratories throughout the world. This group of antibiotics has since become the subject of a highly complex terminology. It appears that in every screening program initiated with actinomycetes, some form of actinomycin was first isolated, thus confirming the dictum of Haeckel that "ontogeny repeats phylogeny." This antibiotic has not as yet become an important chemotherapeutic agent, due to its high toxicity, although recently it has received renewed interest because of its potential antineoplastic activities.

Among the other antibiotics to have been isolated from cultures of actinomycetes prior to 1943, it is sufficient to mention the following.

Proactinomycin<sup>24</sup> was later separated into several chemical entities. This group of substances was obtained from a culture of an organism at that time believed to be a member of the genus *Nocardia* (*Proactinomycetes*), but more recently it was found to belong to the genus *Streptomyces*.

Streptothricin, a basic, water-soluble compound, not too toxic, and active against both gram-positive and gram-negative bacteria, was isolated from a strain of *Streptomyces lavendulae* in 1942.<sup>25</sup> The isolation of this antibiotic may be considered of great historical significance, since it pointed a way to a new type of desirable antimicrobial agent. Had no other, less toxic compound been soon isolated, it would have definitely found a place in chemotherapy. It appeared to supplement penicillin in its activity on different microorganisms. Since streptothricin was active not only against gram-positive and gram-negative bacteria but also against fungi and acid-fast bacteria, it may be considered as the first "broad-spectrum" antibiotic, if this term is to be used in a truly etymological sense.

It so happens that the term "antibiotic spectrum" was introduced about that time for characterizing the individual antibiotics. Who would have dreamed, 15 or 16 years ago, that this term would in time split off into such an array of spectra, as "wide," "broad," "narrow," "medium," and "stubborn."

Once the screening procedures were well understood, once proper methods of chemical isolation and purification of antibiotics were developed, and once certain types of compounds came to be recognized, it was logical to expect that each newly isolated substance should possess properties somewhat superior, either in regard to activity or toxicity, to those known previously. Among the preparations obtained from cultures of actinomycetes prior to 1943, streptothricin appeared to possess the most desirable properties, except that it still had a certain delayed toxicity to experimental animals. It was logical, therefore, that the search should be directed for similar substances, but less toxic. In September of that year, or just 15 years ago, we succeeded in isolating a new antibiotic,<sup>26</sup> streptomycin, from a fresh culture of an organism first studied in our laboratory in 1915 and designated at that time as *Actinomyces griseus*.<sup>27</sup> This species designation should be recognized as being *sensu* Waksman and Curtis and not Krainsky. Since the genus *Streptomyces* was created<sup>28</sup> only five months previous to the isolation of this antibiotic, it was logical that the genus should impart its new name to the new antibiotic.

Streptomycin proved to be an excellent chemotherapeutic agent. Not only did it supplement penicillin in its activity upon resistant gram-positive bacteria, but it was also active, in low concentrations, upon gram-negative and acid-fast bacteria. It thus came to deliver the first blow to the ancient foe of the human race, tuberculosis, as well as to numerous diseases caused by gram-negative organisms.

Streptomycin opened the floodgates for the study of the production of antibiotics by actinomycetes. New compounds were now isolated in rapid succession. Efforts were also made to modify chemically the known antibiotics, notably streptomycin, thus yielding dihydrostreptomycin, a substance that appeared at first to have certain more desirable physical and toxicological properties than streptomycin itself.

#### CHLORAMPHENICOL AND THE TETRACYCLINES

It did not take very long before new antibiotics useful as chemotherapeutic agents were isolated from cultures of actinomycetes. Most important of these were chloramphenicol, isolated from a culture of *Streptomyces venezuelae*, soon followed by chlortetracycline from *Streptomyces aureofaciens*, and somewhat later by oxytetracycline from *Streptomyces rimosus*. More recently, tetracycline itself was discovered both in the broth of the organisms and as a reduction product of chlortetracycline. These antibiotics took up the battle against infectious diseases where penicillin and streptomycin left off. They proved to be effective against those infections that either became resistant to the last two drugs or were not sufficiently sensitive to them; they were also effective on a variety of infections not acted on by those antibiotics, such as the rickettsial diseases and diseases caused by larger viruses.

Tremendous screening programs for new antibiotics were now in operation. It may be said truthfully that, following penicillin, all the major antibiotics that have found an application in human and animal chemotherapy came from cultures of actinomycetes. Whereas only two decades ago barely a dozen or so laboratories throughout the world were concerned with these organisms, they were now being isolated, largely from the soil, in literally thousands of laboratories. The capacity of actinomycetes to produce antimicrobial substances was being investigated at an ever increasing rate. Just a few of the subsequent developments will be examined further.

The screening programs for new antibiotics produced by actinomycetes now took on a definite pattern. Some were directed toward a search for substances that would possess properties similar to those of streptomycin, especially its antituberculosis activities. Others were concerned largely with antifungal agents. Still others were searching for antiviral and antineoplastic agents. The most important of the newly discovered substances, belonging to the glucoside type of antibiotics, were the neomycins, the erythromycins, the novobiocins, and related compounds.

The neomycins comprise a series of chemical substances ranging from neomycin itself, made up of two isomeric entities, B and C, and its derivative neamine, and, continuing through different degrees of complexity, to catenulin, framycetin, and, more recently, kanamycin. The high auditory toxicity of neomycin prevented its use in tuberculosis treatment, but it has gradually found an important place in oral and topical therapy. The streptothricin and the streptomycin groups continued to attract attention, with the result that a whole chain of interlocking compounds with a tremendous vista of chemical interrelationships were unravelled.

The rapid development of resistance to penicillin among the staphylococci has led to an intensive search for antibiotics that would possess strong antistaphylococcal properties. A large number of substances were isolated, varying in degree and range of activity, toxicity, and solubility, as well as in other desirable properties. The most important of these are the erythromycins, carbomycin, leucomycin, and spiramycin. Numerous other basic glucosidic antibiotics have been isolated, but it is still difficult to say what place they will eventually find in chemotherapy.

The further search for antistaphylococcal agents yielded other antibiotics that possessed additional desirable properties. These include greater persistence in the blood, effect upon bacteria that have become resistant to other antibiotics, and desirable activity upon infections, such as brucellosis, that have not responded well to previously known antibiotics. These newly discovered agents include novobiocin, oleandomycin, vancomycin, and others.

Numerous additional compounds have been isolated from cultures of actinomycetes and found to possess a variety of chemical and biological properties. Only brief mention need be made of them: (1) the polypeptides, comprising viomycin, cinnamycin, amphomycin, cycloserine, and valinomycin; (2) pigmented substances, notably litmocidin, rhodomycin, rhodomycetin, and coelicolorin; (3) sulfur containing compounds, such as thiolutin, thiostrepton, actithiazic acid, and sulfocidin; (4) iron containing compounds, such as grisein; and (5) nitrogen free compounds, such as mycomycin, and various others. Some of these antibiotics are gradually finding an important place in certain forms of therapy.

#### ANTIFUNGAL AGENTS

With the gradual conquest of infections due to bacteria, more and more attention is being paid to disease conditions due to other groups of organisms, including both the more complex forms, such as fungi and protozoa, and the simpler forms, notably the viruses. The search for antifungal agents does not appear to present any serious difficulties. This would appear to be particularly the case for the fungi causing human and animal diseases, as well as plant pathogens and rot-producing fungi. A large number of antifungal antibiotics have already been isolated. Beginning with cycloheximide, a companion antibiotic of streptomycin, and nystatin, one of the first polyene antibiotics that has found an application as a chemotherapeutic agent, nu-

merous new compounds have been isolated. The problems of solubility, stability, and toxicity appear to be of paramount importance in determining their potential usefulness. The polyenes alone, ranging from the tetra- to the octaenes, comprise numerous compounds, especially the tetraenes (nystatin and antimycin) and the heptaenes (candididin, ascocin, trichomycin, amphotericin B, and candidin), some of which have already found practical applications.

#### ANTIVIRAL AND ANTINEOPLASTIC AGENTS

As soon as it was recognized that various groups of microorganisms have the capacity to inhibit the growth of pathogenic bacteria and fungi, especially when some of them began to find a place in chemotherapy, the idea was suggested that certain organisms might possibly have the capacity to form similar agents that would inhibit the growth of and even destroy animal and plant viruses. Although many such substances have now been isolated, and although some appear to have a definite repressive effect upon the small viruses, none has as yet found practical application. One of the difficulties involved is a lack of a quick and reliable testing procedure. It was at first thought that bacterial viruses or phages could be used for this purpose. Unfortunately, it was soon found that agents active on bacteriophages have no effect on the true animal or plant viruses. Apparently the spectrum of activity is as specific and as variable for antiviral agents as is the action of antibiotics upon bacteria and fungi. Some of the antiviral substances also have an effect upon bacteria, but others do not. Some are active against certain viruses and not upon others. The tendency to ascribe the origin of certain types of cancer to viruses has added greater significance to this type of investigation.

Among the antiviral agents obtained from cultures of actinomycetes, one may mention ehrlichin, abikoviromycin, and luridin. As far as we are aware at present, the most significant part of these isolations is the increase of our information about the possible existence of similar mechanisms that may prove in time to be effective in the treatment of infectious diseases caused by viruses.

The problem of formation of antineoplastic agents by microorganisms in general and by actinomycetes in particular dates back many decades, when it was observed that certain bacterial infections, such as erysipelas, have the capacity to suppress the growth of certain tumors. An extensive literature has accumulated dealing largely with the effect of bacteria or their products (Coley's toxins) upon tumor growth. Actinomycetes began to receive consideration only when their role as producers of antibiotics came to be recognized. It may be mentioned here, as an aside, that one of the most debated "tumor cures" in recent years has an *Actinomyces* underlining. The peculiar assumption has been made that "lumpy jaw" of cattle, due to *Actinomyces bovis*, may be a tumor; a vaccine was therefore produced from an unknown *Actinomyces* culture, which was said to cause the disease; this particular preparation was said to cure tumors in man. Both assumptions and conclusions seem to be farfetched.

By a most peculiar coincidence, it was found that the first true antibiotic preparation isolated from an *Actinomyces* culture, namely, actinomycin, had certain anti-tumor properties. This led to a renewed interest in this antibiotic of which numerous forms have now been obtained, varying in toxicity and activity. Among the other antineoplastic agents, it is sufficient to mention azaserine, DON, puromycin, and sulfactin, isolated in this country; sarcomycin, carzinophillin, and mitomycin, isolated

in Japan; and a number of others. The search is now going on at an ever-increasing rate.

## OUTSTANDING PROBLEMS IN THE FIELD OF ANTIBIOTICS

The tremendous developments in a field of science that may be said to have begun only about 20 years ago and that has since attained a stature of tremendous importance to human health and human economy, have left open many scientific problems that require extensive study.

First of all, there is the biogenesis of the antibiotics. It is known that certain chemical substances added to the medium will favor the production of specific antibiotics or of different forms of a given antibiotic, as in the case of the penicillins and the actinomycins. It is further known that specific environmental conditions favoring one type of metabolism over another will not only increase the yield of a particular antibiotic, but will often change its chemical composition. Some antibiotics are found largely in the mycelium of the organisms rather than in the culture broth. The mechanism of formation of the antibiotics, the enzyme systems involved, and their role in the nutrition of the organisms producing them still remain largely obscured. In the case of streptomycin, for example, the formation of 5000 units of this antibiotic per ml. of broth actually means a yield of 5 Gm. of the compound per liter. If one stops to think that about 30 to 50 Gm. of the organic substrate is used per liter of medium and that only about one quarter to one third of the nutrients consumed are converted to mycelium, one must postulate the fact that about one third to one half of that mycelial growth is made up of the antibiotic. It places the latter beyond the scope of an intermediate or a waste product of metabolism. It must be considered rather as a storage product, such as starch in wheat or in potatoes or inulin in sweet potatoes. Thus, the biogenesis of the antibiotic is closely tied to its role in the metabolism of the organism. Since each organism produces more than one antibiotic or several related chemical substances possessing antimicrobial properties, the question arises as to the extent that the metabolism of the organism can be modified to favor the formation of one type of antibiotic in preference to another.

Then there is the problem of the mode of action of the antibiotic. We still know relatively little concerning the selective action of antibiotics on different bacteria. If we could tie it to the particular staining properties of the sensitive organisms, we could postulate certain hypotheses. But we could hardly do that, since many antibiotics are active against various gram-positive and gram-negative bacteria. Some are active only on fungi and not on bacteria and actinomycetes. Just what connection is there between the nature of the organism and its sensitivity to a particular antibiotic?

This leads us to the problem of development of resistance. Not to be overlooked is also the phenomenon of development of dependence of the organism on the presence of a particular antibiotic. What do we really know about the activity of the antibiotic and the metabolism of the sensitive and resistant bacteria?

The genetics of actinomycetes has recently received some attention and deserves far more consideration. The problem of mutagenicity may be greatly modified. Is a culture pure when one can isolate from it so many distinct variants, or is it a mixed population to begin with?

The classification of the genus *Streptomyces* is another important problem that should receive careful consideration, since it is from members of this genus that all the important antibiotics have so far been isolated. Two International Congresses

just held in Europe devoted considerable attention to this subject. It is essential from the taxonomic point of view and even from the patent point of view that classification be done properly. To describe each fresh culture as a new species, as some of our friends in this country and abroad have done, or to describe a fresh isolate as a new species and then include an important organism already described before within such a species, is scientifically inaccurate, logically unsound, and hardly proper from any point of view. Worse yet is to give a new name without even an accompanying description.

Whether we retain the Actinomycetales as an independent order or place them with the Eubacteriales under distinct families,\* we must recognize the existence of the following four important categories within each family: the genus, the group (series, section), the species, and the variety (strain). Ten distinct genera have now been established. Some 400 species have already been described, and some 600 names can be listed among the incompletely described forms. Numerous varieties have been or should have been created. The value of the group (series) characteristics for classification and identification purposes still remains to be established.

There are a host of other problems that might be worth considering. It is sufficient to mention the role of the antibiotic in the growth and nutrition, in the survival, if you please, of the organism producing it. This also brings into the picture the significance of ecological relationships, biotic phenomena, and the question of competition among microorganisms in natural substrates.

#### AN OUTLOOK

You would now ask, what of the future? From the large number of *Actinomyces* cultures examined, antibiotics isolated,† and the great variety of chemical compounds and biological activities thus obtained, one would think that the limit may already have been approached. To one who has devoted more than four decades of his life to the study of actinomycetes, to one who has searched for them in numerous soils throughout the world—in peat bogs and in composts, in lakes and in seas—it all appears as a mere beginning. Stop to think that a single gram of soil taken outside of this window may yield, when plated out on suitable media, a million or more colonies of actinomycetes, representing numerous species with many metabolic potentialities. How many of these can one investigator, even if surrounded by a team of assistants, test in a single lifetime? How many of the biochemical mechanisms can one study, with all due allowance for the cultural variations of the organisms, the effect of the composition of the medium upon the products formed, and the scientific preparation of the investigator? All I can say is that there is still much left to be done. Many mechanisms are still to be uncovered. To those of you who are only starting on this search, I can only say: Go ahead, the field is yours, the future is full of promise, the harvest is bound to come!

#### BIBLIOGRAPHY

1. WAKSMAN, S. A.: The soil as a source of microorganisms antagonistic to disease-producing bacteria, *J. Bact.* 40:581–600, 1940.

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\* Before I leave this subject of taxonomy of the actinomycetes, I must emphasize again that all the recent evidence points to their closer relationship to the bacteria than to the true fungi.

† Of some 30 antibiotics now in practical use, at least 25 are produced by actinomycetes.

2. National Research Council. Committee on Chemotherapeutics and Other Agents. Clinical investigation of streptomycin. Report No. 2, May 31, 1946.
3. Food and Drug Administration. Federal Security Agency. Specifications for streptomycin; standards of identity, strength, quality and purity. Effective July 15, 1946.
4. ANON.: Antibiotics, Ann. New York Acad. Sc. 48:31-218, 1946.
5. WAKSMAN, S. A.: Antibiotics of actinomycetes, with special reference to certain challenging problems, notably the development of bacterial resistance. Conference on Antibiotic Research. Antibiotics Study Section, National Institutes of Health, January 31 and February 1, 1947, Washington, D. C.
6. GASPERINI, G.: Recherches morphologiques et biologiques sur un microorganisme de l'atmosphère, le *Streptothrix Foersteri* Cohn, Ann. Microgr. 10:449-474, 1890.
7. LIESKE, R.: Morphologie und biologie der strahlenpilze (actinomyceten). Gebruder Borntraeger, Leipzig, 1921.
8. GRATIA, A., AND DATH, S.: Propriétés bacteriolitiques de certaines moisissures, Compt. rend. Soc. de biol. 91:1442, 1924.
9. ROSENTHAL, L.: La lyse des bacilles diphtériques effectuée par un *Streptothrix*, Compt. rend. Soc. de biol. 93:77-79, 1925.
10. WELSCH, M.: Influence de la nature du milieu de culture sur la production de lysines par les Actinomycètes, Compt. rend. Soc. de biol. 126:244-246, 1937.
11. WELSCH, M.; GHUYSEN, J. M., AND CASTERMANS, A.: The multiplicity of bacteriolytic agents in actinomycetes, Proc. 3rd Intern. Cong. Biochem., 1955, p. 413-415.
12. BORODULINA, U. S.: Vzaimootnosheniya pochvyennikh aktinomycetov i *B. mycoides*, Mikrobiologiya 4:561-586, 1935.
13. NAKHIMOVSKAYA, M. I.: Antagonism myezhdoo aktinomycetami i pochvennymi bakteriyami, Mikrobiologiya 6:131-157, 1937.
14. KRASSILNIKOV, N. A., AND KORENIKO, A. I.: Bakteritsidnoye vyeshyestvo aktinomycetov, Mikrobiologiya 8:673-685, 1939.
15. KRIS, A. E.: O lyzozyme y aktinomycetov, Mikrobiologiya 9:32-38, 1940.
16. ALEXOPOULOS, C. J., AND HERRICK, J. A.: Studies in antibiosis between bacteria and fungi—III. Inhibitory action of some actinomycetes on various species of fungi in culture, Bull. Torrey Botan. Club 69:257-261, 1942.
17. GREIG-SMITH, R.: Contributions to our knowledge of soil fertility; the action of certain microorganisms upon the numbers of bacteria in the soil, Proc. Linn. Soc. N. S. Wales 42:162-166, 1917.
18. WAKSMAN, S. A., AND HUTCHINGS, I. J.: Associative and antagonistic effects of microorganisms: III. Associative and antagonistic relationships in the decomposition of plant residues, Soil Sc. 43:77-92, 1937.
19. MILLARD, W. A., AND TAYLOR, C. B.: Antagonism of microorganisms as the controlling factor in the inhibition of scab by green-manuring, Ann. Appl. Biol. 14:202-216, 1927.
20. McCORMACK, R. B.: The associative action of some species of actinomycetes. Thesis, Cornell University, 1935.
21. KEN KNIGHT, G.: Studies on soil actinomycetes in relation to potato scab and its control, Mich. Agric. Exper. Sta. Tech. Bull. 178, 1941.
22. TIMS, E. C.: An actinomycete antagonistic to a *Pythium* root parasite of sugar cane, Phytopathology 22:27, 1932.
23. WAKSMAN, S. A., AND WOODRUFF, H. B.: *Actinomyces antibioticus*, a new soil organism antagonistic to pathogenic and non-pathogenic bacteria, J. Bact. 42:231-249, 1941.
24. GARDNER, A. D., AND CHAIN, E.: Proactinomycin: A bacteriostatic produced by a species of *Proactinomyces*, Brit. J. Exper. Path. 23:123-127, 1942.
25. WAKSMAN, S. A., AND WOODRUFF, H. B.: Streptothricin, a new selective bacteriostatic and bactericidal agent, particularly active against gram-negative bacteria, Proc. Soc. Exper. Biol. & Med. 49:207-210, 1942.
26. SCHATZ, A.; BUGIE, E., AND WAKSMAN, S. A.: Streptomycin, a substance exhibiting antibiotic activity against gram-negative and gram-positive bacteria, Proc. Soc. Exper. Biol. & Med. 55:66-69, 1944.
27. WAKSMAN, S. A., AND CURTIS, R. E.: The actinomycetes of the soil, Soil Sc. 1:99-134, 1916.
28. WAKSMAN, S. A., AND HENRICI, A. T.: The nomenclature and classification of the actinomycetes, J. Bact. 46:337-341, 1943.

## Introduction of Doctor Chester S. Keefer

DR. MARTI-IBAÑEZ (Moderator). Thank you, Dr. Waksman, for your magnificent address. Once again we have had the opportunity of admiring your gifts as a philosopher of science, dedicated to wresting from Nature her most intimate secrets. Such an abnegated task redeems a man from being—to quote your own poetic words in your book *My Life with the Microbes*—"a speck of dust."

In the history of medicine there is a group of remarkable physicians who have excelled in organization and leadership. To such group belongs the conquerors of malaria and yellow fever. There is a great physician among us who belongs also to that group. He undertook the gigantic task of organizing the distribution and clinical use of penicillin and streptomycin during the heroic period of these two drugs, when every gram was precious and meant not only a human life but also the future of a vast research project of incalculable curative promise.

I refer to Dr. Chester Keefer, who, during the Second World War, directed the Committee of the Division of Medical Science, National Research Council, and developed the clinical trials for the two first great antibiotics, penicillin and streptomycin. The world owes a debt of gratitude to Dr. Keefer for his work when penicillin was only a hope and streptomycin was only a promise.

With great pleasure I introduce Dr. Keefer, Director of the Robert Dawson Evans Memorial Hospital.

# The Impact of Antibiotics on American Medicine

CHESTER S. KEEFER

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I am honored to be invited to address this Sixth Annual Symposium on Antibiotics. I want to say that these conferences serve a very useful purpose in the transmission of current information and the assessment of trends and perspectives concerning a variety of anti-infective agents. They provide an opportunity for all who are concerned with the advancement of knowledge of infectious diseases and chemotherapy to come together and share their information. It is a real forum in which diversity of opinion and disputes are expressed. Sometimes confusion is added to complex problems. It is, however, tempered by an air of tolerance and patience.

So, I congratulate those who organize and sponsor these conferences for their interest in bringing together people who are interested in one of the most exciting of all areas in medicine. You are shortening the lag period between discovery of new knowledge and its application for the benefit of mankind.

The discovery, development, and use of antibiotics for the crusade against infectious diseases is clearly one of the most revolutionary factors in American medicine, yes, indeed, in global medicine. The antibiotics have altered profoundly the way in which millions of people are treated when ill. They have changed medicine and its practice in all of its aspects almost beyond recognition. These revolutionary changes in medicine have created new possibilities for health and longevity that would have seemed unbelievable even as recently as 30 years ago. This revolutionary force in global medicine, in the field of antibiotics as well as other anti-infective agents, is advancing with amazing swiftness. Since the widespread use of penicillin, we have been making, in the space of years, advances whose past analogues required decades or even centuries. The lead time, the period between discovery and application, has been shortened.

## LEAD TIME

In a recent report by the Commission on Social and Economic Conditions at Mid-Century, a recommendation was made that some attention should be given toward shortening the lag period between the discovery of new knowledge and its application for the benefit of mankind and for better living.

It is a matter of record that, in 1754, James Lind in his *Treatise on the Scurvy* showed to what scurvy is due and advised its treatment, as we do today, by lemon juice. The time scale for the effects of this disclosure, now called "lead time," was as follows. First of all, the Lords of the British Admiralty, concerned at the existence of an agency for the destruction of seamen more effective than the enemies' guns and with the deference for hygiene and human effectiveness of all military bodies, adopted Lind's recommendations for the British Navy after an interval of 40 years, hence the name "Limey" or "Lime Juicer" for a British sailor. However, as Wilfred Trotter tells us in his *Art and Science in Medicine*, it was 150 years before physiology and biochemistry discovered the existence of vitamin C. Thus, it took the Navy 40 years and scientists a century and a half for one of the

clearest and most direct hints from medicine to produce an effect in the world of science.

Such phenomena in the natural history of knowledge are no reproach to science in the past. We have come a long way since 1754 because intimations in medicine today do not take long to reach and inspire the biochemists and physiologists. The familiar facts are submitted to experimental analysis. Our biochemists and other biological scientists are acutely sensitive to direct inspiration by medicine on the one hand, and, on the other, our young men in medicine are more adept and better prepared to make full use of exact experiment and measurement. Gradually, the barriers between the search for new knowledge, without respect to usefulness and the application of new knowledge, are being lowered and destroyed. Medicine is taking the direct attack on its own day-to-day problems. We cannot do this, however, without having some scientists work on the very limit of medical vision. The usefulness of useless knowledge must never be overlooked.

#### IMPACT OF ANTIBIOTICS

The impact of antibiotics upon global medicine has had a tremendous beneficial effect upon all nations of the world. In a recently published economic report, 282 pages in length with another 78 pages of appendices on antibiotic manufacture, there appears a statement that there is no way to measure exactly the contribution of antibiotics to the improved public health situation or the cumulative effects of that improvement on national productivity.

The Bureau of Economics of the Federal Trade Commission admits that the antibiotic industry is important to the public health and welfare, and that antibiotic substances are more potent against a variety of diseases than any drugs previously known, and that other substances produced by antibiotic manufacture have unique value as food and feed supplements in human and animal nutrition. They also state in their resolution that there is a broad public interest in the availability at reasonable prices of antibiotic drugs as well as in continuing research and continuing incentives, for the discovery and development of new uses and new antibiotics under the private enterprise system.

In short, this report was concerned with an investigation of the antibiotic industry because, in the opinion of the Federal Trade Commission, such an investigation would be, as they say, in the public interest.

This report demonstrates that the pharmaceutical industry has produced products that are of inestimable value to mankind, and the record shows that these agents have emerged in a free society and in an environment in which profit incentives and patent protection are important. The desire to make a contribution to the general health and welfare has been the dominant motive. All of this has been in the public interest and for the public benefit. The discovery of new antibiotics and their production has expanded the medical research effort; it has aided in raising the standard of living and health of people; and it has stimulated greater interest in preventive medicine. Moreover, it has stimulated greater demands for improved treatment of infectious diseases when prevention fails. The health professions have had great difficulties in keeping up with public demands for new and better health products. However, I submit, our research workers, our teachers, our clinical investigators, and our industries have been meeting the challenge in a magnificent way.

During the next few days, we will be hearing about a variety of new agents and

their potential place in therapeutics. The impact of these agents will be demonstrated in the future.

It is well to pause from time to time and take stock, to assess our present position, consolidate our gains, and plan for the future. At least it is essential that we ask questions concerning the problems.

While much has been learned about infection, much remains. Where are we at mid-century?

In reflecting upon the subject of infectious diseases and their management by antimicrobial agents, we are dealing with diseases in which the cell poisons are produced by microorganisms living and reproducing in the body. Today, we maintain and require all of the methods of prevention of infections and disease initiated by Pasteur, but we have moved a step further with the introduction of modern chemotherapy. While it is still as necessary as ever to prevent infection as well as disease, with the discovery of antimicrobial agents, we are able to deal with many diseases and microbes more effectively. The attempt to do this has provided an enormous stimulation to the study of the direct action of specific chemicals on microorganisms and on their hosts, man, animals, plants. While the original motive in the discovery of antibiotics was the conquest of infectious diseases in man, the studies have extended to problems of growth and nutrition of animals, the prevention of diseases in animals, the preservation of food, the prevention of disease in plants, and the prevention of death and infections following radioactive poisons.

Discovery of antibiotics has stimulated new research in the broad field of microbiology, genetics, biophysics, biochemistry, pharmacology, and clinical medicine—yes, in all of the biological sciences. Their application to agriculture and animal diseases has stimulated new research in the field of nutrition and the development of the world's food supply.

As physicians we can say that antibiotics have: reduced fatality rates in many diseases; reduced the total duration of many diseases; prevented complications in many diseases and prolonged life in others; prevented certain diseases, such as rheumatic fever; and relieved great suffering.

In the social and economic fields antibiotics have created a new industry, and they have added useful years to life.

The reasons that the costs of antibiotics are so low today are that they can be produced in quantity and in volume, and the need and demand for these products for the promotion of health and well-being is very great. Antibiotics have changed the public attitude toward infectious diseases. They have reduced the total costs of illness caused by many diseases. It is always difficult and, in many instances, impossible to relate the costs of an illness to a dollar value. In any consideration of this problem, it is necessary to take into account the total costs of an illness and medical care, rather than approach the problem from the point of view of the high cost of drugs or any other single item. When one examines the total expenditures for personal health services today, we find that drugs and medical supplies account for only a fraction of the total costs. Other items that need to be considered are professional and hospital care, loss of income from illness, and costs of insurance coverage. The problem, nevertheless, is not simple but it is important.

#### THE NEEDS OF THE FUTURE

It is generally agreed that the two major problems in bacterial infections are staphylococcal diseases and gram-negative bacillary sepsis.

At present, there is no antibiotic, and it is unlikely that there will be a single antibiotic, that will solve all of the problems of staphylococcal infections. There is a pressing and urgent need for broad critical research. This research should include a search for new knowledge of the bacteriology, epidemiology, and pathogenesis of these infections. The problems of staphylococcal infections cannot be solved by searching for new and better antibiotics alone. The research must be extended to include the determination of serological and bacteriophage types, the measurement of drug resistance, the quantitative estimation of the various components and products of the staphylococci, and their capacity to invade tissues. Moreover, the need for more basic information concerning the factors that undermine host resistance and the reactions of immunity are essential.

In the continuing search for new antibiotics that will attack microorganisms, we need to learn more about how they work in destroying bacteria. While some advance has been made, it is far from being complete. I submit that this is by far the most important task of all. It is the most difficult, but it may be the most rewarding. It can lead to much better scientific prospecting for new antibiotics. If it were possible to learn more about the mode of attack of a chemical molecule on a bacterium or a virus, it should be possible to design a molecule that works as well or better. At present, there is some evidence that the efficiency of antibiotics is due to the molecule of the antibiotic being very but not quite like that of the normal food required by the bacterium. Once it is ingested or taken in by the microbe, it alters the metabolism, and changes the growth characteristics.

Also, by studying the mode of action, we might well learn why organisms become resistant to the action of antibiotics, and why they may persist and fail to reproduce when they are in a dormant stage and yet are susceptible to the action of antibiotics in their growth phase.

The whole problem of bacterial mutations needs to be explored with vigor, because one of the waves of future research in chemotherapy and bacteriology will be a deeper and more profound study of the genetics of bacteria.

Another problem that has emerged from the widespread use of antibiotics is the uncovering of some new diseases and the centering of attention upon diseases that failed to respond to the antibiotics. Some of these diseases have increased in frequency, or at least in prominence. What is not too clear is the part the antibiotics have played in this shift of emphasis and in frequency of disease. For example, wound infections were commonly due to the hemolytic *Streptococcus* and the *Staphylococcus*, rarely to gram-negative bacilli. Today, the bacteriological flora of wounds has shifted so that the *Staphylococcus* and gram-negative bacilli are dominating the picture. The hemolytic *Streptococcus* has been eliminated or greatly reduced. Thus, the question needs to be raised, what, if anything, has happened to the host that permits organisms that were commonly not present to make their appearance and thrive.

#### THE PROBLEM OF GRAM-NEGATIVE BACILLARY SEPSIS

The widespread use of antibiotics has centered attention upon the diseases caused by gram-negative bacilli and, especially, those microbes causing diseases of the intestinal and urinary tracts. In the aggregate, these diseases are important, and some progress has been made in their control. Of the enteric infections, it can be said that we are in a better position to control typhoid fever when prevention fails than we were before the days of chloramphenicol. Moreover, many of the

shigellae infections can be treated more effectively since the use of the sulfonamides. The *Salmonella* group continue to remain a problem because our currently available chemotherapeutic agents are ineffective. The same is true for most strains of *Bacillus proteus* and *Bacillus pyocyaneus*.

The problems concerned with *Escherichia coli aerogenes*, the *Bacillus mucosus capsulatis*, and the paracolon group continue to be numerous and deserve comment.

First, many gram-negative bacilli live within the body in peaceful coexistence from birth till death and never cause disease. On occasions, these organisms that have remained dormant will be able to invade tissues and produce necrosis, and they do so when there is a breakdown or rupture of the normal defense mechanism or when the tissue or body metabolism is altered, so that the normal barriers for bacterial invasion of tissues are undermined. We have come to recognize with increasing frequency a shift in the bacterial flora of the throat and the intestinal tract following the use of antibiotics and, also, a striking shift in the flora in respiratory, wound, and urinary tract diseases following the use of anti-infective agents. It is difficult to escape the suggestion that these anti-infective agents not only alter the bacterial flora through their action on bacteria, but they probably alter tissue cellular metabolism so that microorganisms may flourish in an environment that was unfavorable for them without antibiotics. All of the existing experimental evidence available today suggests that certain gram-negative bacilli behave more like animal tissue cells toward many types of antiseptics than do other microorganisms. This view may provide an explanation for the fact that attempts to find chemotherapeutic agents effective against such organisms as the *Salmonella* have presented such great difficulties.

In the search for new anti-infective agents, we need more research based upon sound scientific values and basic prospecting of the factors influencing bacterial growth and metabolism. The practical achievements of chemotherapy so far have come from the utilization of agents that interfere with the metabolism of microorganisms. However, it should not be overlooked that Dubos and his colleagues were the first to demonstrate that it is possible to alter the pathological course of certain organisms, such as the type III pneumococcus, through the enzymatic destruction of its capsule and its products. The search for more information and for enzymes that are specific for broad classes of bacteria may yield products of great value.

So, in the future attack on microbes, we need to utilize the skills of chemists, biologists, geneticists, and physicists. The search must be for basic facts and not merely for seeking cures for specific diseases.

Some questions to be answered are what changes and alters a bacterium so that it is no longer harmed by an antibiotic; also, why do susceptible organisms persist and fail to reproduce when they are in the dormant stage and yet are susceptible to the action of an antibiotic in their growth phase?

#### SUMMARY

It can be said that the discovery, development, production, and application of antibiotics have had a great beneficial effect upon treatment of disease. New problems have come into sharper focus, and gaps in our knowledge have been exposed. The needs for the future are challenging: the means of controlling gram-negative infections, drug-resistant strains of staphylococci, and fungal and viral diseases are all important.

As we learn more about the metabolism of bacteria and their inheritance and the factors controlling their growth and death, we will be in a stronger position to prevent disease following infection and to prevent infection. In all of these studies, we must not overlook the host but must study man as well as the microbes. The question here is: What are the local and general metabolic factors in man that undermine resistance so that infection progresses to disease?

While antibiotics have had a tremendous impact upon global medicine so far, we can look forward to further changes that will improve our capacity to promote health and prevent disease. The trends are all in the direction of progress.

## Introduction of Doctor Harry F. Dowling

DR. MARTI-IBAÑEZ (Moderator). Thank you, Dr. Keefer, for your magnificent oration, which, besides inspiring us about the future, has turned us into witnesses of the growth and development of antibiotics in those days when it was a source of anguish to make a decision as to *when*, *how* and *to whom* antibiotic treatment should be given, and yet you with great insight and impartiality mapped out a wide clear path for these vital drugs.

I am now going to present a true clinical investigator. In medicine, a researcher's life is often closely linked to that of new drugs and new methods. So it is with Dr. Harry Dowling, Professor of Medicine at the University of Illinois, who became, we might say, the "test pilot" for almost every antibiotic developed after that day when broad-spectrum antibiotics were introduced. On that memorable day not only was the spectrum of anti-infection drugs broadened, but also the philosophic concept of treating whole groups of clinical syndromes even before their etiological cause had been identified. Dr. Dowling.

# The History of the Broad-Spectrum Antibiotics

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A bare 10 years ago the word "antibiotic" meant, practically, either penicillin or streptomycin. These antibiotics were producing spectacular effects in certain diseases, yet their limitations had already become obvious. Successful in the coccal diseases and tuberculosis, they were useless in typhoid fever, the rickettsial and viral diseases, and many of the infections caused by gram-negative rods. Resistance to penicillin was beginning to be recognized as a cause of failure in therapy of staphylococcal infections, although this problem had not assumed its present magnitude. Such was the picture when the broad-spectrum antibiotics were born.

The developments of the first of these antibiotics, chlortetracycline and chloramphenicol, showed astonishing parallels. In the same year, 1939, Lederle Laboratories and Parke, Davis and Company began their studies on antibiotics. In 1943, each of them found the man who was to discover the fungus that was the source of a new antibiotic. In that year, Dr. Benjamin Duggar's retirement from the Professorship of Botany at the University of Wisconsin catapulted him into the leadership of Lederle Laboratories' team to search for a new antibiotic. In 1943, also, Parke, Davis and Company established a research grant at Yale University to enable Dr. Paul Burkholder of the Department of Microbiology to screen soil samples for promising microorganisms.

In 1945, Duggar found the *Streptomyces aureofaciens*. Under the direction of Malcolm and SubbaRow, research on *S. aureofaciens* produced the antibiotic that was at first called Aureomycin. At about the same time, Burkholder sent a promising culture of *Streptomyces venezuelae* to the Parke, Davis Research Laboratories, where investigators, under the direction of Kamm, Stimpert, and Sweet, were able to obtain chloramphenicol. Clinical trials of both drugs followed quickly.

The effectiveness of chloramphenicol in rickettsial infections of animals, observed both by the Parke, Davis investigators and by Smadel's group at the Army Medical School, stimulated both groups to use it in rickettsial diseases of man. In December, 1947, Payne of Parke, Davis and Company first gave chloramphenicol to patients with typhus fever in Bolivia. All 16 who received the antibiotic recovered. At almost the same time, Smadel and Ley observed similar recoveries in patients with typhus fever in Mexico. Because scrub typhus was one of the unsolved problems in military as well as civilian medicine, Smadel looked around the world for a suitable spot in which to test this promising new antibiotic. Arrangements were soon made with Lewthwaite in Malaya, and here Smadel and Woodward began their now classical investigations on the treatment and prophylaxis of scrub typhus. While they were treating their first patients, they gave chloramphenicol to 2 Malaysians from a rubber plantation that had been the source of many cases of scrub typhus. One patient got well promptly and proved to have had scrub typhus; the other did not improve immediately, and the course of his illness evolved so that it resembled typhoid fever more than scrub typhus. When laboratory tests also indicated the presence of typhoid fever, the decision was made to continue chloramphenicol therapy, even though it was in short supply and even though in vitro studies against

*Salmonella typhi* had not been too promising. Within four days the patient was practically afebrile, and, when an additional 9 patients responded as promptly, Woodward and Smadel knew they had an effective drug for typhoid fever. While these excellent results were being obtained in Malaya, a group of investigators under the leadership of Pincoffs was demonstrating the effectiveness of chloramphenicol in Rocky Mountain spotted fever. These successes in the rickettsial diseases and typhoid fever were reported in 1948. Chloramphenicol was approved by the Food and Drug Administration in January, 1949, and was introduced to the market in March of that year. It was not until the latter part of 1949 and 1950 that the effectiveness of chloramphenicol in other diseases began to be reported.

In contrast to what might be called the flank attack of chloramphenicol on the infectious diseases, the producers of chlortetracycline delivered a frontal attack on the entire field of infectious diseases from the very start. *S. aureofaciens* was isolated from the soil in 1945, the first animal experiments were done in the following year, and the crystalline substance first obtained in 1947. The antibiotic was first given to patients in January, 1948. By heroic efforts, the laboratories managed to keep several groups of clinicians, in hospitals from Boston to Texas, furnished with sufficient amounts of the drug for patients with a wide variety of diseases. The first clinical investigators included Anigsten, Braley, Dowling, Finland, Long, Schoenbach, and Wright. By July 21, 1948, the accumulated experience justified a symposium at the New York Academy of Sciences, at which successful results were reported in bacterial infections caused by pneumococci, streptococci, gonococci, and meningococci, and in typhoid fever and bacillary dysentery, as well as Rocky Mountain spotted, Q, and typhus fevers among the rickettsial diseases and lymphogranuloma venereum among the viral diseases. A report of this conference and several other clinical papers on chlortetracycline were published in 1948. Furthermore, to establish this year as an important landmark in antibiotic history, chlortetracycline was approved by the Food and Drug Administration on October 20, 1948. It was introduced to the market on December 1, 1948, the first of the broad-spectrum antibiotics to be marketed.

During the years 1948 to 1950, nearly every clinical investigator in the field of infectious diseases was actively extending his experience with chlortetracycline and chloramphenicol. It is remarkable how few mistakes were made in the early clinical evaluation of these antibiotics. At first, some observers thought that one or both of them were effective in herpes zoster, infectious mononucleosis, and viral hepatitis, but these false trails were rapidly abandoned. Today, perhaps the only infectious disease in which the effectiveness of chlortetracycline and chloramphenicol is in doubt is primary atypical pneumonia; this doubt is the result of the scarcity of cases in the past few years.

In 1950, two important events occurred. In 1949, the *Streptomyces rimosus* had been isolated in the laboratories of Chas. Pfizer and Company after several years of searching. Soon afterward, this company developed a method of producing oxytetracycline from it. By utilizing the experiences of others, they were able to shorten their timetable so that they could introduce this new antibiotic to the market by March, 1950. At a symposium at the New York Academy of Sciences in June, 1950, reports were made on a large number of cases of various illnesses treated.

The other significant event in 1950 was the report by Volini and his associates of granulopenia and both granulocytic and erythroid maturation arrest in the bone marrow of a few patients who had received chloramphenicol. This went almost unnoticed at the time, but suddenly a number of reports of blood dyscrasias ap-

peared. Following a nationwide survey by the Food and Drug Administration and on recommendation of an *ad hoc* committee of the National Research Council, the medical profession was warned that blood dyscrasia might follow the use of chloramphenicol. This event initiated the second phase in the use of broad-spectrum antibiotics—from enthusiastic and sometimes uncritical use to doubt, suspicion, and, sometimes, complete rejection. The tetracycline group of antibiotics did not escape this wave of distrust completely, because their administration was also accompanied by side reactions which, although less serious, were often distressing. At first, oxytetracycline and then chlortetracycline were reported to produce diarrhea in some patients. The overenthusiastic administration of these drugs to surgical patients and the long-continued administration to patients in whom the indications were questionable, and in whom, therefore, the indications for discontinuance were not clear-cut, undoubtedly increased the frequency of these side effects. The attitude of wariness that this distrust engendered had a salutary effect. Physicians began to use the tetracyclines more carefully, and when their confidence in chloramphenicol was restored, they likewise administered it more judiciously.

Careful consideration before a broad-spectrum antibiotic was given was made necessary both by the fear of untoward reactions and by the appearance of resistant forms of staphylococci and gram-negative rods. From the very first it had been observed that certain microorganisms were not affected by the broad-spectrum antibiotics in vitro or in vivo. As these drugs were used more extensively, the proportion of resistant forms increased until in some species, such as *Staphylococcus aureus*, the majority of strains were resistant to the concentrations that could be expected from therapeutic doses. At first, chloramphenicol-resistant strains were appearing as well as strains resistant to the tetracyclines, but, when the use of chloramphenicol fell off so rapidly, the proportion of resistant forms not only failed to increase but even appeared to decrease. It remains to be seen what will happen now that chloramphenicol is being used extensively again.

It had been recognized for some time that the microbial spectra of chlortetracycline and oxytetracycline were identical. When, in 1953, the structural formula of tetracycline was found to be similar to those of chlortetracycline and oxytetracycline, except for the elimination of a molecule of chlorine or a hydroxy radical, respectively, the similarities in action were explained. After tetracycline was found to have an identical therapeutic spectrum, it almost completely displaced its analogues, because of superior absorption from the gastrointestinal tract, ease of penetration into the cerebrospinal fluid, and lower incidence of gastrointestinal complications following its administration. The present spate of articles on the effect of adjuvants upon the absorption of tetracycline from the intestine appears to be a mere tempest in a teapot (or shall we say an inkpot, since the number of inches of advertising space utilized seems to be the measure of the success of an adjuvant). The study of Sweeney and his associates<sup>1</sup> on the inhibitory action of calcium phosphate and the neutralization of calcium by certain adjuvants appears to have clarified the picture.

It has now been ten years since the first broad-spectrum antibiotic, chlortetracycline, was offered for sale. What is the status of these antibiotics today? Figure 1 shows the amounts produced each year from 1949 to 1957, inclusive.<sup>2</sup> The downward trend, beginning in 1953 and continuing in 1954, resulted from a reduction in the use of chloramphenicol following the announcement that depression of hemopoiesis sometimes accompanied its administration. The subsequent increases have attended the introduction of tetracycline and a resurgence in the use of chlor-

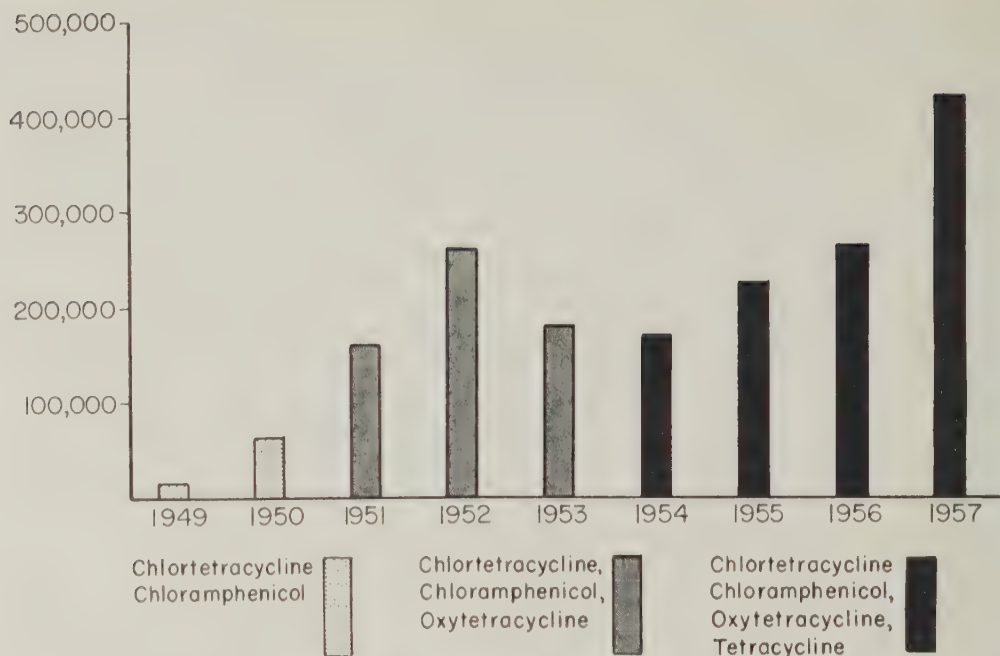


FIG. 1. The production of broad-spectrum antibiotics in kilograms is illustrated.

amphenicol. In 1957, 402,400 Kg. or 443 tons of broad-spectrum antibiotics were produced, most of which were tetracycline.

In a study made during 1955 and 1956, Kinney<sup>3</sup> found that 23 per cent of a group of 586 nurses had received one of the tetracyclines at some time in her life. Nolen and Dille<sup>4</sup> reported that among the inhabitants of a small community in South Dakota, 65 per cent had received one of the tetracyclines and 23 per cent had received chloramphenicol during a period of five years, from 1952 through 1956. One can truthfully say that the broad-spectrum antibiotics have entered thoroughly into the life of the American people.

What are they used for? The field of therapy may be divided into: (1) Diseases for which these antibiotics are superior to all other drugs. These include diseases caused by rickettsiae, larger viruses, and *Hemophilus influenzae*, and brucellosis, pertussis, typhoid fever, and other *Salmonella* infections; (2) infections in which certain strains of bacteria may be more susceptible to the broad-spectrum antibiotics, while others may be more susceptible to other antibiotics. Among these are infections with staphylococci, coliform bacteria, and other gram-negative rods; and (3) infections in which not only the broad-spectrum antibiotics but also one or more of the other antibiotics are efficacious for all strains of the infecting organism. These include infections with pneumococci, group A streptococci, meningococci, gonococci, shigellae, amebae, and *Treponema pallidum* and certain mixed infections, such as peritonitis, following the rupture of a viscus and bronchopulmonary supuration.

Prophylactic uses have been fewer: (1) the broad-spectrum antibiotics have been shown to be effective in suppressing scrub typhus; (2) they can be used to lower the number of bacteria in the gut preoperatively and in ulcerative colitis or cirrhosis of the liver; and (3) they will reduce the frequency and severity of acute pulmonary infections in patients with chronic bronchitis and bronchiectasis.

There are two additional large fields of usefulness. The first is the growth-promot-

ing effect of the tetracyclines upon animals. This is presumably the result of suppressing the growth of microorganisms in the intestines. The second is the preservation of foods. The use of chlortetracycline for this purpose was approved on November 30, 1955. This antibiotic is peculiarly suited to this purpose because it is less stable in the presence of heat than are the other broad-spectrum antibiotics. The quantity needed to preserve food will thus be greatly decreased or completely destroyed in cooking.

All of the uses of the broad-spectrum antibiotics that I have listed have been of great practical value. In addition, investigations of the clinical administration of the antibiotics have added much to our knowledge. We first wrestled seriously with, and I hope clarified, the problem of analogues among antibiotics in working with the tetracyclines. This helped us to understand and evaluate analogues of other antibiotics as they appeared. Likewise, we encountered the problem of combinations of antibiotics when the tetracyclines or chloramphenicol were used along with penicillin. The problem of superinfection had been present only in slight degree when penicillin or streptomycin was administered; superinfection was thrust upon us forcefully by the broad-spectrum antibiotics, and we had to learn its dangers and how to deal with it.

Most important of all, in my opinion, we have learned, through the production, development, and popularization of the broad-spectrum antibiotics, how industry can contribute more significantly to the common welfare. We have learned how scientists, administrators, and promotional personnel in industry can integrate their efforts with those of investigators in universities and hospitals and with practicing physicians for the good of all. It is interesting that penicillin was discovered in one university hospital and developed in another university, that streptomycin was discovered and developed in a single university, that chloramphenicol was discovered in a university and developed by a pharmaceutical company, and that chlortetracycline was discovered and developed entirely by a pharmaceutical company. The universities broke the path; industry, with its superior resources and its practical objectives, straightened, widened, and paved the path and thus accelerated the rate of progress. The discovery of the first effective antibiotic was made by a single investigator in a small laboratory. Production and clinical application, however, required teamwork between investigators, manufacturers, and clinicians. The story of an antibiotic, from the first tentative observations to the final use in the patient, is a marvelous saga involving thousands of people, each of whom contributes an important bit to the whole.

Man invariably seeks a hero as the symbol of his achievements and as an object for his wonder. The history of the broad-spectrum antibiotics reveals no single hero. Rather, it is the history of many men and women thinking together, planning together, working together, studying, communicating, and achieving together. The accomplishments of these men and women may well rank among man's greatest cooperative endeavors, and each one who participated, no matter how small his share, can be proud to have taken part. "The real and legitimate goal of the sciences," said Francis Bacon, "is the endowment of human life with new inventions and riches."<sup>5</sup>

But there is a warning here also. The path that is first blazed through the forest, then straightened and broadened into a road, and finally paved so that it will bear the heaviest traffic, eventually comes to be so crowded and confused, so filled with automobiles maneuvering to squeeze ahead of each other that no one makes any progress. When the broad-spectrum antibiotics were first produced, the path was

faint and forbidding except to those with imagination. Today one produces new antibiotics as he learns to drive a car—by imitating others. If everyone drives along these same roads, we will be able to treat infections no better ten years from now than we do today. Let us look further. Even when the forest seems to be impenetrable, there are hints of trails that await exploration. The future history of antibiotics will be written by those who have the imagination and the courage to search out the new paths.

#### ACKNOWLEDGMENTS

The author wishes to thank Dr. J. H. Williams for information regarding the early history of chlortetracycline, Drs. H. E. Carnes and T. E. Woodward for information on chloramphenicol, and Dr. J. H. Kane for information on oxytetracycline.

#### BIBLIOGRAPHY

1. SWEENEY, W. M.; HARDY, S. M.; DORNBUSH, A. C., AND RUEGSEGG, J. M.: Absorption of tetracycline in human beings as affected by certain excipients, *AM & CT* 4:642–656, Oct., 1957.
2. WELCH, H.: Personal communication.
3. KINNEY, J. R.: The prevalence of antibiotically treated people, *Ann. Int. Med.* 47:71–77, July, 1957.
4. NOLEN, W. A., AND DILLE, D. E.: Use and abuse of antibiotics in a small community, *New England J. Med.* 257:33–34, July 4, 1957.
5. BACON, F.: *Novum Organum*, Chicago, Henry Regnery, 1949, p. 42.

## Closing Remarks to the Historical Session and Presentation of the Soil Samples of the Broad-Spectrum Antibiotics

DR. MARTI-IBÁÑEZ (Moderator). Thank you, Dr. Dowling, for your excellent address, which enabled us to follow the labored journey, all the way through dark valleys to the sunlit peaks, of clinical research in broad-spectrum antibiotics.

The expository historical section of the Symposium, encompassing two memorable anniversaries in the history of antibiotics, has come to an end. We shall now proceed with the presentation to the Smithsonian Institution of historic soil samples of Aureomycin, Terramycin, and Chloromycetin.

This has already been a memorable Symposium, for rarely in medical history has a discoverer been afforded the opportunity of critically assessing his own discovery. Today, we have had the good fortune to listen to some of the greatest figures in antibiotic medicine objectively reassessing their past discoveries and achievements.

Dr. Henry Welch will now take over the chairmanship of this session, and will introduce Dr. Carey, who will deliver the original sample of Aureomycin that initiated the history of this drug.

My long, intimate, and cherished friendship with Dr. Welch demands that I do not embarrass him, whose natural modesty I know only too well, with the eulogy he so richly deserves. Besides, he would begrudge me the time spent on him. But I will say, and I know you will agree with me, that Dr. Welch is a great example of that rare combination: man of science, organizer, and humanitarian. He has dedicated his life not only to antibiotic research, but also to spreading antibiotic culture through his writings and lectures and through symposia he himself has organized. In his great athletic chest there beats an intensely human heart, towered by a magnificent scientific mind. With pride and affection, I turn this meeting over to Dr. Henry Welch.

**Dr. Henry Welch.** The original soil samples of Aureomycin, Chloromycetin, and Terramycin will now be presented to the Smithsonian Institution.

We are most fortunate to have with us as representatives from the Smithsonian Institution, Dr. Robert P. Multhaupt, Head Curator of the Smithsonian Institution, Department of Science and Technology; Dr. George B. Griffenhagen, Curator of the Division of Medical Sciences, and Dr. John B. Blake, Assistant Curator of that division.

The broad-spectrum antibiotics have brought about a tremendous reduction in morbidity and have saved the lives of thousands since they were made available for clinical use. The first of the broad-spectrum drugs discovered was chloramphenicol; then followed chlortetracycline and oxytetracycline, and finally tetracycline through dechlorination of chlortetracycline. Chlortetracycline was the first broad-spectrum antibiotic made available for general clinical use. More than 870,000 pounds of these antibiotics were produced for human and veterinary medicine in 1957 and a like amount was produced for agricultural uses, such as for

animal feeds, preservation of raw poultry, and crop sprays. Thus production has risen in less than 10 years from none to more than 1,700,000 pounds.

It is quite appropriate that the soil samples from which the organisms responsible for these most important drugs were isolated be preserved in the Smithsonian Institution, rich in history itself and custodian of the national collections of history, science, and art.

Dr. Benjamin W. Carey, Medical Director, Lederle Laboratory Division, American Cyanamid Co., will present the Aureomycin soil sample; Dr. John Ehrlich, Laboratory Director in Antibiotic Research, Parke, Davis & Co., will present the Chloromycetin soil sample; and Dr. Ernest M. Weber, Director of Biochemical Research, Chas. Pfizer & Co., will present the Terramycin soil sample.

**Dr. Benjamin W. Carey** (Lederle Laboratories Division, American Cyanamid Co.): Thank you, Doctor Welch, for your warm introduction.

My task this morning is an unusual one. Unusual, in the sense that I have come to Washington to present a tiny bottle of Missouri soil to the Smithsonian Institution. Taken literally, it might appear to be a peculiar thing to do.

However, from this soil a team of Lederle scientists, led by 76 year old Dr. Benjamin Duggar, isolated the organism *Streptomyces aureofaciens*. In turn, the organism yielded the golden antibiotic, Aureomycin. Introduced just 10 years ago, it has helped physicians throughout the world to save the health and lives of millions.

The soil itself came from the campus of the University of Missouri. It was taken from Sanborn Field, which is an experimental area run by the University's Agricultural School. Doctor W. A. Albrecht, a long-time friend of Doctor Duggar, sent the soil sample to Lederle along with some 60 others in answer to a request from Doctor Duggar.

This soil was among the thousands of samples that our research staff screened. The initial success of the antibiotic isolated from it, in controlling experimental infections in the laboratory, was followed by intensive clinical investigation, described by Dr. Dowling, before being made available to the medical profession.

Incidentally, Doctor Duggar went to Sanborn Field several years after his discovery and, with Doctor Albrecht, took another soil sample from the very spot that had yielded *Streptomyces aureofaciens*. This time they found no interesting molds.

This is the soil that did it. The inscription on the bottle is extremely simple, considering the importance of its contents: "Soil Sample 67, Columbia, Missouri, taken from plot #23, continuous timothy, no fertilization, silt loam. I wish to present Mrs. Louise M. Ellis, of Suffolk, Virginia, who is in the audience, the first patient with Rocky Mountain spotted fever that was treated with Aureomycin. At that time, Mrs. Ellis was a young girl living in Cleveland, North Carolina."

At this time, I would like to call Dr. Robert P. Multhauf, of the Smithsonian Institution to the platform. As Head Curator of the Institution's Department of Science and Technology, Doctor Multhauf will officially receive the soil sample.

Doctor Multhauf, it is a privilege to hand this historic soil over to you. We trust that it will be a valuable addition to your collection.

**Dr. John Ehrlich** (Parke, Davis & Co.): This is a bit of soil from a mulched stubble field near Caracas, Venezuela. It was from a pinch of soil from this Venezuelan field that Paul Burkholder isolated a novel and remarkable antibacterial microbe later named *Streptomyces venezuelae*. This was the organism with which a Parke-Davis team first prepared the antibiotic now known as Chloromycetin. It is my pleasant duty to present this memorable bit of soil to our National Museum.

**Dr. Ernest M. Weber** (Chas. Pfizer & Co.): On behalf of Chas. Pfizer & Co., Inc., it gives me great pride to present to the Smithsonian Institution a portion of the original soil sample that yielded the Terramycin producing culture, *Streptomyces rimosus*. We be-

lieve that there can be few scientific exhibits anywhere in this world which can contain the life-saving potentials inherent in this deceptively simple portion of soil.

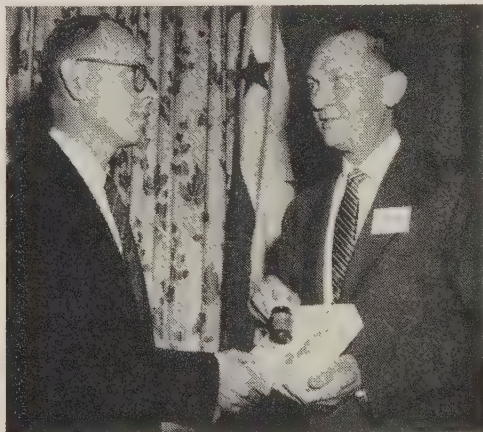
A great opportunity and challenge to explore and study this sample was given to our company. Since all mankind benefits through scientific developments of this magnitude, we can do no less than to dedicate this original soil sample to the world-wide community of knowledge.

There can be no better custodian of this historic sample than the Smithsonian Institution.

**Dr. Robert P. Multhauf** (Smithsonian Institution): The Smithsonian Institution belongs to the people of the United States. It seems appropriate therefore that these samples of soil from which were developed three important antibiotics should belong to the Smithsonian.

This occasion also gives me the opportunity to remind you that the public is not willing merely to be cured by these drugs. It also wants to understand, so far as possible, what the development of antibiotics is "all about." Our medical museum, and those elsewhere, are trying to do this job. The acquisition of specimens of genuine historic importance, such as these, help us to do it. We extend our thanks to Dr. Benjamin W. Carey and to Lederle Laboratories, Division of American Cyanamid Co., to Dr. John Ehrlich of Parke, Davis & Co., and to Dr. Ernest Weber of Chas. Pfizer & Co.

Presentation of original soil samples to Dr. Robert P. Multhauf, Head Curator, Department of Science, Smithsonian Institution. (*Right*) Dr. Benjamin W. Carey, Lederle Laboratories Division, American Cyanamid Co., presents the original Aureomycin soil sample. (*Below*) The original Chloromycetin soil sample is presented by Dr. John Ehrlich, Parke, Davis & Co.



(*Right*) Presentation of the original Terramycin soil sample by Dr. Ernest Weber, Chas. Pfizer & Co., Inc.

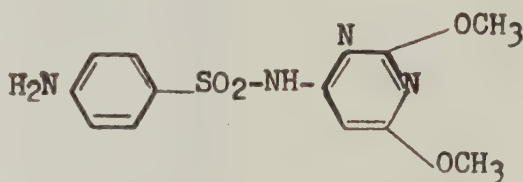


# A New Long-acting Antibacterial Sulfonamide, 2,4-Dimethoxy-6-Sulfanilamido-1,3-Diazine: A Comparative Study

WILLIAM P. BOGER

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The modern chemotherapeutic era was heralded by the introduction of the azo dye, Prontosil, and, although this drug can now be found only in an occasional pharmacy, the sulfonamide drugs that came afterward hold a very significant place among the drugs that are useful in the treatment of infections. The earlier compounds, which made the first great strides in our attack against infectious disease, have largely been superseded and the search has gone steadily forward to discover compounds that are equally effective antibacterially, possess less toxicity, and have pharmacologic properties that make them more useful in meeting specific treatment needs. The new compound 2,4-dimethoxy-6-sulfanilamido-1,3-diazine (Madribon\*) appears to have some of these useful attributes. Following precedent in the contraction of the cumbersome chemical designation of sulfonamide drugs, this compound will for convenience be hereinafter referred to as sulfadimethoxine. The compound is tasteless and odorless with the following structural formula.



The solubility of sulfadimethoxine, as is true for all sulfonamides, is dependent upon the *pH* of the solvent. In water, within the *pH* range of 3.1 and 5.7, the solubility lies between 4.6 and 7.2 mg./100 ml. In the *pH* range of 6.2 to 7.06 the solubility lies between 13 and 58 mg./100 ml. Animal toxicity data indicate freedom from any unusual physiological effects and the only item of passing interest was the observation that rather uniformly at dosage levels of 50 mg./Kg. or more, there was a goiterogenic effect. The potential of a number of sulfonamides to exert an anti-thyroid effect with production of goiter is well known. Tests for the antibacterial effectiveness of sulfadimethoxine have been carried out and as measured by the influence of the drug upon the course of experimental mouse infections produced with the *Streptococcus*, the penumococcus, the *Staphylococcus*, and a variety of other organisms usually susceptible to sulfonamide treatment, this new drug seemed to be comparable to other currently available sulfonamide therapies. Preliminary administration of this compound to patients suggested that the drug was rapidly absorbed following oral administration and was slowly excreted.<sup>1</sup>

The rapid absorption after oral administration, the animal studies indicating therapeutic efficacy and the preliminary indications of slow excretion in man, seemed to justify the following comparative studies.

\* The trade name of Hoffmann-La Roche for sulfadimethoxine is Madribon.

TABLE I  
Comparison of Sulfadimethoxine and Sulfisoxazole  
Plasma Concentrations and Urinary Recoveries  
after Single 2 Gm. Oral Dose

Pt.	Sex	Age	Wt.		Hours after dose						Total mg. recovery in urine			
					½	2	4	6	8	24	0-2	2-4	4-6	6-8
<i>Sulfadimethoxine</i>														
H.F.	F	42	152	Free	2.8	14.4	17.7	18.2	18.0	14.3	4.86	8.71	22.50	20.67
				Total	3.4	15.4	18.9	20.0	20.2	16.7	4.75	8.51	24.20	23.40
W.M.	F	27	112	Free	1.6	10.4	20.3	22.5	22.6	16.7	3.95	19.75	37.10	39.00
				Total	1.6	10.9	21.2	24.0	24.8	19.1	4.75	20.30	39.40	44.30
H.C.	M	49	125	Free	6.1	12.6	17.0	19.8	19.4	15.9	8.55	16.05	26.00	27.80
				Total	6.2	13.6	18.3	21.9	21.2	17.9	8.85	16.35	28.65	31.45
N.M.	M	23	168	Free	2.9	8.3	13.3	13.1	13.3	12.1	8.33	24.60	26.10	44.85
				Total	3.4	8.7	14.4	14.3	14.6	14.1	9.63	28.60	31.60	54.00
L.J.	F	37	148	Free	3.1	12.7	13.6	14.4	14.0	12.1	4.57	24.80	25.15	29.78
				Total	3.5	13.4	15.0	15.9	15.2	14.2	5.40	28.38	32.00	34.40
M.MC	F	33	128	Free	4.2	14.0	15.7	17.5	16.1	12.1	9.90	26.95	38.10	13.42
				Total	5.0	15.2	17.3	19.8	19.4	14.8	10.88	32.80	47.10	17.30
Average				Free	3.5	12.1	16.3	17.6	17.2	13.9	6.61	20.14	29.16	29.25
				Total	3.9	12.9	17.6	19.3	19.2	16.1	7.37	22.49	33.82	34.14
<i>Sulfisoxazole</i>														
H.F.	F	42	152	Free	16.4	26.9	25.6	21.0	19.0	7.1	131.7	187.0	184.0	108.2
				Total	19.2	32.4	28.6	24.9	24.2	12.7	152.2	224.1	246.0	155.0
W.M.	F	27	112	Free	7.6	12.0	23.8	22.4	20.3	5.1	34.0	167.2	181.0	214.0
				Total	9.6	13.9	25.6	25.9	24.2	8.1	37.6	189.0	251.5	296.0
H.C.	M	49	125	Free	30.0	26.9	22.8	17.6	14.8	5.1	115.5	159.5	328.0	239.6
				Total	36.8	32.6	26.3	22.8	21.3	7.2	142.8	215.8	385.8	281.8
N.M.	M	23	168	Free	8.8	17.9	14.8	10.8	7.7	1.2	194.6	236.8	490.5	234.6
				Total	9.2	19.2	16.1	12.5	9.1	1.9	195.0	236.0	497.5	271.3
L.J.	F	37	148	Free	1.2	21.0	19.0	15.0	11.4	2.1	258.3	418.0	167.0	158.8
				Total	1.6	21.2	20.2	16.7	12.5	2.6	252.0	428.0	200.0	190.0
M.MC	F	33	128	Free	12.7	15.7	15.2	15.4	11.4	1.5	272.0	408.0	311.0	183.2
				Total	12.4	15.6	17.2	17.9	12.7	2.4	251.0	371.5	350.0	236.0
Average				Free	12.8	20.0	20.2	17.0	14.1	3.7	167.6	262.7	276.9	189.8
				Total	14.8	22.5	22.3	20.1	17.3	5.8	171.7	277.4	321.8	238.3

#### METHODS

All determinations of sulfonamide concentrations were done by the Bratton-Marshall technique employing each of the particular sulfonamides as its own standard for the estimations. All reporting is in terms of plasma concentrations as opposed to blood concentrations; the reasons for this have been previously discussed.<sup>2</sup> It should be pointed out that plasma concentrations are approximately two times blood concentrations. Drugs were administered in the same doses, 2 Gm., to fasting patients. Venous blood specimens were drawn into heparinized syringes, the plasma separated by centrifugation and the samples frozen until assayed.

#### COMPARISON WITH SULFISOXAZOLE

Six patients, 4 women and 2 men, ranging in age between 23 and 49 years and varying in body weight from 112 to 148 pounds, were studied as their own controls in a crossover pattern. On the first day, 3 patients received a single oral 2 Gm. dose of sulfadimethoxine and 3 other patients received a similar dose of sulfisoxazole.\* At ½, 2, 4, 6, 8, and 24 hours after administration, blood samples were obtained and assayed for sulfonamide content. During the first eight hours of observation, frac-

\* The trade name of Hoffmann-La Roche for sulfisoxazole is Gantrisin.

tional urine samples were obtained 0 to 2, 2 to 4, 4 to 6, and 6 to 8 hours, respectively. One week later (a minimum period of time necessary for the clearing of the very slowly excreted sulfonamides) the administration of drugs was reversed and the same pattern of blood sampling and urine collections followed. The individual and average data are presented in table I. Sulfisoxazole is rapidly absorbed from the gastrointestinal tract and reached a peak concentration in the plasma at two hours, whereas the peak concentration of sulfadimethoxine is achieved slightly later, four hours or even six hours after administration. The concentrations of the sulfisoxazole decline over a 24 hour period to a value of approximately 4 mg./100 ml., whereas sulfadimethoxine is quite slowly excreted and a high order of plasma concentration is maintained over the entire 24 hour period of observations. Still another point of difference is the amount of drug that is excreted into the urine during an eight hour period. The average recovery of sulfadimethoxine was approximately 5 per cent, whereas that of sulfisoxazole was nearly 50 per cent.

#### COMPARISON WITH SULFAETHIDOLE AND SULFAMETHOXYPYRIDAZINE

Nine patients, 2 men and 7 women, ranging in age between 23 and 47 and ranging in body weight from 107 to 170 pounds, were studied as their own controls on three separate days in a crossover pattern. The group of 9 patients was divided into three subgroups of 3 patients each. On any one day of study 3 patients received each of three sulfonamide preparations under investigation. All drugs were administered to patients in a fasting condition and heparinized venous blood samples were drawn from the antecubital vein at  $\frac{1}{2}$ , 2, 4, 6, 8, and 24 hours after the oral administration of 2 Gm. single doses of the various compounds. Fractional urines were obtained over eight hour periods at 0 to 2, 2 to 4, 4 to 6, and 6 to 8 hours, respectively. In table II may be seen the individual and average data with regard to the plasma concentrations and urinary recoveries of the three sulfonamides.

It may be again observed that the peak concentration of sulfadimethoxine is achieved in the plasma at approximately four hours and excellent concentrations are maintained over a period of 24 hours. In the case of sulfaethidole\* and sulfamethoxy-pyridazine,† the peak plasma concentrations are achieved a little earlier and are observed at two hours.

The concentrations achieved after sulfaethidole are not maintained at an equally high level for the entire 24 hour period, but nevertheless significant amounts, approximately 6 mg./100 ml. are observed at the end of this time.

The comparability of the plasma concentrations of the new compound sulfadimethoxine and sulfamethoxy-pyridazine are reflected in the almost identical urinary recoveries observed over the eight hour periods studied, namely, 6 per cent for sulfadimethoxine and 10 per cent for sulfamethoxy-pyridazine. Nearly 50 per cent of sulfaethidole is excreted in the same eight hour period and hence it can be clearly stated that sulfaethidole is much more rapidly excreted than either of the other two compounds.

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\* The trade name of Smith, Kline & French Laboratories for sulfaethidole is Sul-Spansule. The drug used in this study was an especially prepared 0.5 Gm. tablet physically comparable to tablets of sulfadimethoxine.

† The trade name of Lederle Laboratories Division, American Cyanamid Co., for sulfamethoxy-pyridazine is Kynex. The trade name of Parke, Davis & Co. for sulfamethoxy-pyridazine is Midicel.

TABLE II

*Comparison of 3 Sulfonamides in the Same 9 Patients after Single Oral 2 Gm. Dose*

Pt.	Sex	Age	Wt.		Hours after dose						Total mg. recovery from urine			
					½	2	4	6	8	24	0-2	2-4	4-6	6-8
Sulfadimethoxine														
E.G.	F	20	114	Free	2.4	13.6	20.0	19.5	19.5	16.0	7.6	14.0	25.1	24.0
				Total	2.8	14.4	22.0	21.1	21.1	18.4	12.3	17.7	31.0	31.0
J.W.	M	41	167	Free	5.0	17.4	15.0	17.3	17.3	14.3	14.0	27.1	52.5	50.2
				Total	5.6	18.9	20.4	19.2	19.7	16.3	18.0	34.9	68.0	66.5
H.F.	F	47	107	Free	4.3	10.2	14.7	16.0	14.3	12.1	4.0	12.5	27.2	27.9
				Total	4.7	11.1	16.3	17.5	16.1	13.6	5.8	15.0	33.8	32.8
M.S.	F	37	134	Free	1.8	16.3	19.2	16.9	16.8	15.0	1.0	—	76.7	43.6
				Total	1.9	18.2	21.0	18.3	18.2	17.3	1.2	—	100.0	55.0
D.S.	F	23	122	Free	2.9	11.2	15.2	15.6	14.8	12.6	3.8	24.4	16.0	38.3
				Total	3.8	12.5	17.1	17.5	16.8	14.7	3.7	29.4	20.1	48.9
J.R.	F	37	157	Free	4.0	8.3	11.2	10.8	10.4	11.3	9.7	28.3	32.7	31.7
				Total	4.5	9.4	11.9	11.5	11.3	13.1	11.2	32.0	37.2	35.9
H.S.	F	23	170	Free	0.4	5.0	20.5	22.1	20.5	12.8	2.0	—	21.8	83.4
				Total	0.4	5.8	23.1	24.4	22.8	15.1	3.1	—	25.0	105.0
R.G.	M	24	150	Free	2.8	11.3	17.9	18.9	19.8	14.4	6.3	18.5	19.4	25.5
				Total	2.9	11.8	19.2	20.4	20.8	16.3	10.8	24.2	25.8	35.0
C.C.	F	35	148	Free	3.9	13.0	17.9	17.5	17.0	13.6	8.3	17.8	42.7	29.5
				Total	4.3	13.9	19.4	18.3	19.3	15.4	11.4	21.4	52.4	35.9
Average				Free	3.0	11.8	16.8	17.2	16.7	13.6	6.3	20.4	34.9	39.2
				Total	3.4	13.0	18.9	18.7	18.5	15.6	8.6	24.9	43.7	49.6
Sulfamethoxypyridazine														
E.G.	F	20	114	Free	6.9	25.2	24.8	24.8	24.5	19.8	—	30.0	30.6	28.4
				Total	7.2	26.7	27.9	26.7	26.3	22.4	—	44.4	49.8	47.4
J.W.	M	41	167	Free	7.6	20.2	20.2	18.5	17.1	11.4	19.6	46.6	43.7	47.7
				Total	8.1	21.6	22.6	21.1	19.4	13.9	29.8	81.0	98.7	110.0
H.F.	F	47	107	Free	11.8	18.7	20.4	19.1	18.5	15.5	3.8	20.2	22.1	22.5
				Total	12.1	20.1	21.7	20.8	19.9	17.9	5.3	29.9	36.2	36.0
M.S.	F	37	134	Free	14.9	20.7	20.4	19.1	18.5	15.5	3.8	20.2	22.1	22.5
				Total	16.2	22.6	22.4	22.4	21.5	12.8	4.1	67.2	114.0	110.0
D.S.	F	23	122	Free	7.5	21.6	18.7	19.6	19.1	12.3	15.4	21.5	28.3	30.9
				Total	8.1	22.4	23.8	22.6	21.9	15.7	28.5	58.3	87.5	95.2
J.R.	F	37	157	Free	13.1	20.0	19.1	19.1	19.0	15.0	19.3	34.0	33.0	47.3
				Total	13.2	21.1	19.9	20.6	20.4	16.8	26.4	58.6	57.4	84.0
H.S.	F	23	170	Free	2.4	22.6	20.4	19.0	18.1	10.2	16.9	33.0	37.0	25.4
				Total	2.8	24.5	22.9	21.1	20.8	12.8	25.9	84.2	84.4	60.0
R.G.	M	24	150	Free	4.7	11.6	18.7	18.9	18.1	13.2	5.0	17.7	35.2	26.5
				Total	5.0	12.2	19.9	20.8	20.4	15.8	9.3	35.1	71.5	50.5
C.C.	F	35	148	Free	11.1	19.6	23.8	21.0	19.6	12.5	8.8	22.5	41.4	28.8
				Total	11.8	21.6	26.2	23.5	22.7	15.8	15.4	47.5	95.0	68.5
Average				Free	8.8	20.0	20.7	20.0	19.2	13.5	11.3	28.9	36.5	34.6
				Total	9.4	21.4	23.0	22.2	21.5	16.0	18.1	56.2	77.2	73.5
Sulfaethidole														
E.G.	F	20	114	Free	13.4	23.4	23.7	24.0	22.5	9.1	51.5	151.5	202.5	228.5
				Total	13.9	24.8	25.4	25.6	24.0	9.6	58.0	168.0	218.0	252.0
J.W.	M	41	167	Free	7.0	18.8	20.0	16.6	14.0	6.7	158.2	336.2	318.2	434.7
				Total	7.2	19.6	21.1	17.8	14.7	7.2	170.0	342.0	334.2	483.0
H.F.	F	47	107	Free	11.7	21.8	20.7	17.6	13.6	6.7	45.2	171.5	167.0	180.1
				Total	13.0	23.0	22.1	19.0	15.7	7.2	48.2	189.3	187.3	194.0
M.S.	F	37	134	Free	11.7	20.0	17.6	12.3	8.9	3.2	284.0	714.7	261.4	157.2
				Total	12.9	21.0	18.7	13.1	9.4	3.4	309.0	753.0	332.0	176.0
D.S.	F	23	122	Free	10.8	24.6	20.0	17.0	15.6	6.0	193.6	560.7	217.0	225.6
				Total	10.2	27.0	21.2	18.5	16.7	6.3	220.0	596.0	244.0	251.8
J.R.	F	37	157	Free	8.2	19.4	20.0	16.1	13.6	6.0	135.3	328.8	340.0	383.6
				Total	8.6	20.4	20.8	17.1	15.0	6.7	153.3	355.0	368.0	417.0
H.S.	F	23	170	Free	0.4	5.6	17.7	23.2	18.8	4.0	3.3	98.3	171.6	668.9
				Total	0.4	6.1	18.6	25.4	20.7	4.3	3.6	108.0	180.0	750.0
R.G.	M	24	150	Free	5.6	23.2	26.2	23.2	18.4	5.7	127.7	268.8	324.1	294.4
				Total	6.1	25.0	27.9	25.0	20.3	6.3	140.0	290.0	357.0	346.0
C.C.	F	35	148	Free	10.8	20.4	17.4	14.6	13.2	5.7	47.2	315.5	271.2	211.5
				Total	12.2	21.3	18.6	15.5	14.1	6.3	53.0	352.1	308.0	231.8
Average				Free	8.8	19.7	20.4	18.3	15.4	5.9	114.0	327.3	252.5	309.4
				Total	9.4	20.9	21.6	19.6	16.7	6.4	128.3	350.4	280.9	344.6

TABLE III  
*Diffusion of Sulfadimethoxine into Cerebrospinal Fluid  
after Single 2 Gm. Oral Dose*

2 hours after medication, mg./100 ml.			4 hours after medication, mg./100 ml.			6 hours after medication, mg./100 ml.			12 hours after medication, mg./100 ml.			24 hours after medication, mg./100 ml.		
Pt.	Free	Total	Pt.	Free	Total	Pt.	Free	Total	Pt.	Free	Total	Pt.	Free	Total
1	0.12	0.16	10	<0.10	<0.10	18	0.88	0.94	28	0.61	0.67	38	0.43	0.45
2	<0.10	<0.10	11	<0.10	<0.10	19	0.31	0.32	29	0.20	0.21	39	0.30	0.30
3	<0.10	<0.10	12	0.11	0.12	20	0.13	0.10	30	0.30	0.24	40	0.24	0.24
4	<0.10	<0.10	13	<0.10	<0.10	21	0.15	0.16	31	0.20	0.20	41	1.41	1.67
5	<0.10	<0.10	14	<0.10	<0.10	22	0.18	0.18	32	0.11	0.12	42	0.20	0.23
6	<0.10	<0.10	15	<0.10	<0.10	23	0.17	0.18	33	0.25	0.25	43	0.50	0.52
7	0.10	0.11	16	0.40	0.43	24	<0.10	<0.10	34	0.10	0.12	44	0.33	0.32
8	0.20	0.21	17	0.37	0.39	25	<0.10	<0.10	35	0.33	0.32	45	0.41	0.43
9	0.19	0.10				26	0.10	0.10	36	0.11	0.12	46	0.22	0.22
						27	<0.10	<0.10	37	0.38	0.39	47	0.22	0.21
Av.	0.11	0.12		0.17	0.18		0.22	0.23		0.26	0.27		0.43	0.46

#### DIFFUSION OF SULFADIMETHOXINE INTO THE CEREBROSPINAL FLUID

A diagnostic spinal tap is performed at the time of admission of all patients to our hospital and, accordingly, it is possible by appropriate premedication to determine the extent to which a reference substance diffuses into the cerebrospinal fluid. By a prior test, it was established that the sulfadimethoxine did not interfere with any of the usual diagnostic tests carried out upon the cerebrospinal fluid and, accordingly, groups of patients were premedicated at 2, 4, 6, and 24 hour intervals prior to the performance of spinal puncture. All patients were given single oral doses of 2 Gm. of sulfadimethoxine and cerebrospinal fluid assayed for content of this drug. A total of 47 patients were studied and the results are presented in table III. As early as two hours after administration of the sulfonamide, small but detectable quantities were found in the spinal fluid. The amounts increased slightly as the interval of time between the administration of medication and observation increased. However, it should be noted that at the 24 hour period the average figures are somewhat misleading inasmuch as patient 41 had abnormally high values. If this observation be eliminated and the other 9 patients averaged, the value is little different from those observed at the 6 and 12 hour periods. It should be noted that although both free and total drug was measured in the spinal fluid, the two determinations are practically identical, suggesting that whatever diffusion of sulfonamide occurs is due to diffusion of the free compound.

#### DISCUSSION

It is clear on the basis of the studies reported here that sulfadimethoxine can be characterized as follows: It is rapidly absorbed following oral administration; a high order of plasma concentration is achieved and maintained over a 24 hour period following a single 2 Gm. oral dose; it is conjugated to a very limited extent (approximately 10 per cent); it is slowly excreted into the urine chiefly in the form of free (unconjugated) drug; and it diffuses into the cerebrospinal fluid in small amounts.

Comparisons with other sulfonamides in terms of plasma concentrations and urinary recoveries permit certain general statements. Although sulfisoxazole is somewhat more rapidly absorbed than sulfadimethoxine so that there is a slight dif-

ference in the peak concentrations observed within the first two hours following administration, thereafter sulfadimethoxine maintains concentrations that are distinctly higher than those following sulfisoxazole from the sixth to the twenty-fourth hour after administration. The urinary recoveries covering eight hours after oral administration indicate that approximately 10 times as much sulfisoxazole is excreted into the urine as is the case with sulfadimethoxine.

Sulfaethidole and sulfamethoxypyridazine are absorbed with almost equal rapidity and slightly more rapidly than sulfadimethoxine. Peak concentrations of the same order of magnitude are achieved at approximately the same levels. Sulfadimethoxine produces peaks a little later and at levels not quite so high. Of these three sulfonamides, sulfaethidole is the most rapidly excreted and from the eighth to the twenty-fourth hour plasma concentrations decline. It is for this reason that the presently employed schedules of dosage call for the administration of sulfaethidole twice daily at 12 hour intervals.

Sulfadimethoxine seems to be quite similar to sulfamethoxypyridazine and the differences, if any, must be further defined by future investigation. The present studies were carried out in the same patients in the hope of detecting differences in the metabolic handling of the sulfonamides studied and the findings do suggest that sulfadimethoxine and sulfamethoxypyridazine may be metabolized, at least quantitatively, in slightly different patterns. In this and previous studies<sup>3</sup> sulfamethoxypyridazine was found to circulate in the blood with about 10 per cent of the compound in the conjugated form. Reference to table II shows that this same circumstance obtains with regard to sulfadimethoxine. Similarly, in this and previous work<sup>3</sup> about 10 per cent of an administered dose of sulfamethoxypyridazine was recovered and such a recovery may be regarded as comparable to that for sulfadimethoxine. However, whereas 40 to 60 per cent of the recovered amounts of sulfamethoxypyridazine are in the conjugated form of the drug, only 15 to 25 per cent of the sulfadimethoxine is conjugated. Whether this difference will prove significant cannot be stated at this time, but it is known that the conjugated forms of sulfonamide drugs tend to be less active antibacterially, less soluble, and more toxic. Hence, one might postulate that sulfadimethoxine may prove to be slightly less toxic than sulfamethoxypyridazine and slightly more effective in the treatment of urinary tract infections.

Sulfadimethoxine does diffuse into the cerebrospinal fluid in small amounts. This diffusibility through uninflamed meninges is better than observed for some sulfonamides, but less good than that noted for others.<sup>4-6</sup>

During these studies, sulfadimethoxine has been administered in 2 Gm. single oral doses to 80 persons. Careful inquiry was made with regard to nausea and vomiting, diarrhea, headache, dizziness, back pain, hematuria, crystalluria, and photosensitivity, but none of these symptoms was observed. Clinical use of this new sulfonamide has been too limited to permit any statement beyond those pertaining to toxicity and tolerance of the drug. Oral doses of 1 Gm./day have been administered to 11 patients with urinary tract infections for periods of 7 and 11 days without any evidence of intolerance.

The slow excretion of sulfadimethoxine and the maintenance of high plasma concentrations over extended periods of time offer the potential of widely spaced oral dosage schedules. Such schedules can be of real benefit in the management of patients needing a long therapeutic or prophylactic program of medication, in the handling of uncooperative individuals who swallow medication reluctantly, in pediatric practice where unreliability of oral medication is notorious, and lastly in the handling of large groups of persons as in mass prophylaxis against streptococcal,

menigococcal, or gonococcal infection. The very virtue of this long-acting, new sulfonamide requires full understanding of its pharmacology in order that maximum benefits be realized. An attempt has been made to point out previously<sup>3,5</sup> that sulfonamide drugs differ greatly in their properties and in order to "tailor treatment to therapeutic need," and achieve the "individualization of treatment" which is so desirable, these differences must be recognized. It is disheartening to read such editorial comment as: "The sulfonamides are highly effective in the treatment of pneumococcal pneumonia. The dosage is 6 to 8 gms. daily."<sup>7</sup> Such a statement only contributes to the regrettable tendency to speak generically of sulfonamides and to think of them in terms of a "common dosage schedule." It has been previously emphasized<sup>3</sup> that failure to recognize the pharmacologic differences between sulfamerazine and that of the other sulfapyrimidines resulted in overdosage, serious complications, and finally virtual abandonment of sulfamerazine as a single therapeutic agent. This course of events was not due to a failure of the drug either at the therapeutic level or to a lack of desirable properties, but rather a failure of proper use of the drug.

The foregoing comments regarding sulfamerazine are pertinent to sulfadimethoxine. Its excellent absorption and maintenance of high plasma concentrations, its slow excretion rate, and its antibacterial effectiveness<sup>1</sup> will permit radical departures from the commonly employed sulfonamide dosage schedules. Certainly daily dosage of as little as 1 or even 0.5 Gm. for adults and smaller doses for younger patients seem feasible. Whether this daily dose will be administered as a single or divided doses will be optional. It is mandatory, however, that the dosage schedule of this new and interesting compound be distinguished clearly from that of sulfadiazine, sulfamethazine, sulfisoxazole, and that of dual or triple mixtures of sulfapyrimidines. Unless this is done, it may be anticipated that overdosage with sulfadimethoxine will occur and the compound be blamed for toxicity that is not the fault of the drug itself. Because of the very low renal clearance of this compound and of the similar drug, sulfamethoxypyridazine, they should be used in even smaller amounts (less than 1 or 0.5 Gm./day) in the presence of an elevated nonprotein nitrogen or blood urea nitrogen or in elderly patients in whom a certain amount of renal impairment may be assumed on the basis of aging. Preliminary studies have indicated that as little as 0.25 Gm./day maintain therapeutically adequate plasma concentrations of these sulfonamides when treatment is needed for elderly patients or persons with impaired renal function.<sup>8</sup>

#### SUMMARY AND CONCLUSIONS

The long-acting, antibacterial sulfonamide, 2,4-dimethoxy-6-sulfanilamido-1,3-diazine, referred to in this paper as sulfadimethoxine, has properties that appear to offer the potential of great flexibility of oral dosage schedules for all of the therapeutic and prophylactic uses for which sulfonamides have been found effective.

The drug is rapidly absorbed following oral administration and achieves high plasma concentrations within four to six hours and maintains them above 10 mg./100 ml. for at least 24 hours following single oral doses of 2 Gm. The drug circulates largely in form of free, antibacterially active compound, approximately 10 per cent being in conjugated form. Within eight hours about 6 per cent of an administered dose of sulfadimethoxine is recovered in the urine as compared to 10 per cent for sulfamethoxypyridazine, 50 per cent for sulfisoxazole, and 50 per cent for sulfaethidole (sulfaethylthiadiazole). Also, whereas some 40 to 50 per cent of

the amounts of sulfamethoxypyridazine are excreted into the urine in the conjugated form, only 15 to 25 per cent of the sulfadimethoxine is in this form.

The study of 47 individuals showed that sulfadimethoxine diffuses promptly and moderately well through the uninflamed meninges, into the cerebrospinal fluid.

Within the limits of this study, no side effects from the administration of this compound were observed, but the opinion is expressed that if the dosage of this drug and that of similar long-acting compounds are not clearly distinguished from the much larger daily doses that are required when using the ordinary systemic sulfonamide drugs, toxicity will be encountered which will reflect overdosage and misuse of the agent, rather than a fault of the drug itself.

#### ACKNOWLEDGMENTS

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#### BIBLIOGRAPHY

1. ANON.: Unpublished data supplied by Hoffmann-La Roche, Inc.
2. BOGER, W. P.; STRICKLAND, S. C., AND GYLFE, J.: Comparison of sulfapyrimidines: A triple sulfonamide mixture, sulfisoxazole, and sulfadimetine, *AM & CT* 3:43-47, June, 1956.
3. BOGER, W. P.; STRICKLAND, S. C., AND GYLFE, J. M.: Sulfamethoxypyridazine (Kynex): A new long-acting sulfonamide, *AM & CT* 3:378-387, Nov., 1957.
4. BOGER, W. P.; STRICKLAND, S. C., AND GYLFE, J.: Sulfisoxazole (Gantrisin): Concentration in plasma and diffusion into the cerebrospinal fluid, *J. Am. Ger. Soc.* 5:520-529, May, 1957.
5. BOGER, W. P.: The antibacterial sulfonamides: comparative studies, copyrighted reprint distributed at AMA Clinical Sessions, Philadelphia, Dec., 1957.
6. BOGER, W. P.: The diffusion of sulfonamides into the cerebrospinal fluid: a comparative study, *AM & CT* 6, 1959. In press.
7. ANON.: Chemotherapy of specific infectious diseases of the lower respiratory tract, report of committee on chemotherapy and antibiotics, American College of Chest Physicians, *Dis. of Chest* 33:435-440, April, 1958.
8. BOGER, W. P.: To be published.

# Sulfadimethoxine, a New Long-acting Sulfonamide

## Some Preliminary Clinical and Laboratory Observations in Infants and Children

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A new antibacterial sulfonamide, 4 sulfanilamido-2, 6 dimethoxy pyrimidine, sulfadimethoxine (Madribon\*), has recently been made available for clinical investigation. In animal experimentation, this compound was found to have an antibacterial activity superior to that of sulfisoxazole. In addition, it showed good absorption from the intestinal tract, slow urinary excretion, minimal acetylation, and good solubility in urine.

The purpose of the present investigation was as follows: (1) to determine the optimal dosage of sulfadimethoxine in the pediatric age group; (2) to observe the range in blood levels when repeated doses were given at 24 hour intervals; (3) to compare its blood sulfonamide levels with those of other long-acting sulfonamides, such as sulfamethoxypyridazine and N'-acetyl sulfamethoxypyridazine, in equivalent dosage; (4) to determine the degree of diffusion across the blood-brain barrier; (5) to observe any untoward reactions and compare their frequency with sulfamethoxypyridazine; and (6) to accumulate some preliminary data regarding the efficacy of sulfadimethoxine in susceptible bacterial infections.

The structural formulae of sulfadimethoxine, sulfamethoxypyridazine, and N'-acetyl sulfamethoxypyridazine are shown in figure 1.

### METHODS AND MATERIALS

In the present study, sulfadimethoxine as well as sulfamethoxypyridazine† and N'-acetyl sulfamethoxypyridazine‡ were given on a dose/weight basis, as is customary in pediatric practice. Blood samples were obtained by micromethods. Both blood and spinal fluid levels were determined promptly by a modified Bratton-Marshall technique.<sup>1</sup> For determination of total sulfonamides (free plus conjugated), hydrolysis was carried out on blood filtrates with hydrochloric acid.

Jones and Finland<sup>2</sup> have pointed out that the assaying of paired blood and plasma specimens, following the administration of sulfamethoxypyridazine, indicated that, except in occasional instances, all of the sulfonamide in the whole blood could be accounted for in the plasma and none could be detected in the cellular elements. Thus, the levels in plasma were nearly twice as high as those in whole blood. This has been largely overlooked in accounting for some of the discrepancies in pediatric dosage recommendations that have appeared in the literature thus far. In the present study, no attempt was made to determine the distribution of sulfadimethoxine between plasma and red cells. However, because of the similar chemical structure, one might hypothecate that the same distribution of drug is obtained as with sulfamethoxypyridazine, namely, that little if any sulfadimethoxine enters the red cells. If this were so, whole blood assays would be approximately one-half those of plasma.

\* The trade name of Hoffmann-La Roche for sulfadimethoxine is Madribon.

† The trade name of Parke, Davis & Co. for sulfamethoxypyridazine is Midicel.

‡ The trade name of Lederle Laboratories Division, American Cyanamid Co., for N'-acetyl sulfamethoxypyridazine is acetyl Kynex.

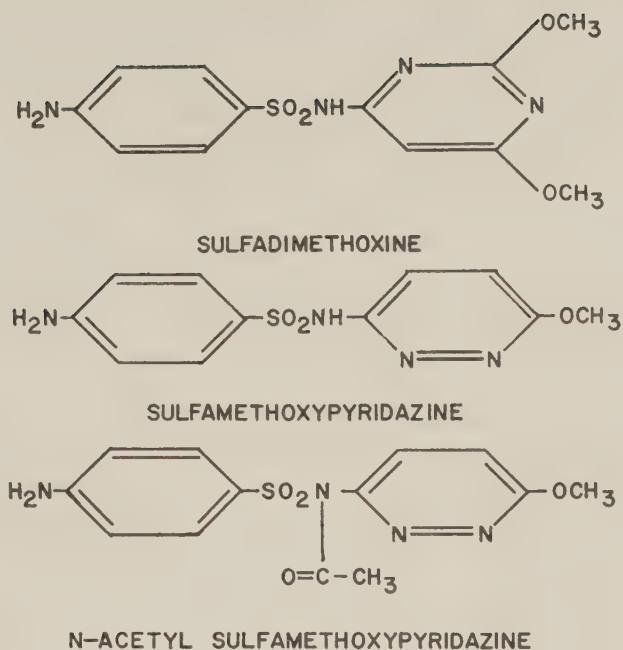


FIG. 1. Structural formulae of three sulfonamides.

As previously noted, all of the assays in the present study were performed on whole blood.

#### RESULTS

*Blood Levels After a Single Oral Dose.* Single doses of sulfadimethoxine of 6.25, 12.5, 25, and 50 mg./Kg. body weight were given to a total of 20 children, varying in age from 3½ to 9 years old and ranging in weight from 15 to 45 Kg. Free and total blood sulfonamide levels were determined at intervals of 2, 4, 8, 24, 48, and 72 hours. For comparative purposes, the same number of children were given equivalent doses of sulfamethoxypyridazine and N'-acetyl sulfamethoxypyridazine, and blood levels were determined at the same intervals.

The resulting free blood sulfonamide levels, in mg./100 ml., were averaged and are presented in table I. They are compared with sulfamethoxypyridazine and N'-acetyl sulfamethoxypyridazine in figures 2 to 5.

As seen in table I, when a single 6.25 mg./Kg. dose of sulfadimethoxine was administered, a peak level of 2.3 mg./100 ml. was achieved at four hours, followed by a slow tapering off to trace levels after 24 hours. Levels obtained with this dose were suboptimal throughout. In figure 2, it may be noted that the same dosage produced somewhat higher levels with sulfamethoxypyridazine and somewhat lower levels with N'-acetyl sulfamethoxypyridazine during the first 24 hours; however, during the next 48 hours, there was close correspondence of the levels of all three

TABLE I  
*Sulfadimethoxine Blood Levels After a Single Oral Dose*

Dose, mg./Kg.	Average blood level, mg./100 ml., hour after dose					
	2	4	8	24	48	72
6.25	1.7	2.3	1.9	1.1	0.8	0.0
12.5	6.2	5.9	5.8	3.0	1.1	0.8
25	6.4	8.5	8.1	5.4	3.2	0.8
50	13.4	14.2	15.4	10.3	5.1	3.4

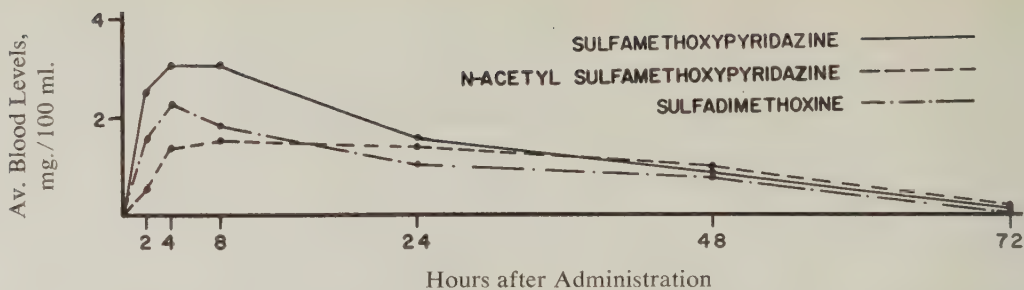


FIG. 2. Comparison of sulfonamide blood levels of three sulfonamides after oral administration of a single dose, 6.25 mg./Kg.

drugs. It is apparent, however, that a 6.25 mg./Kg. dose of all three compounds produces less than adequate blood levels by commonly accepted standards. This is to be especially noted, in view of the fact that some of the dosage recommendations for sulfamethoxypyridazine still call for such a daily dose in the pediatric age group.

From table I, it may be seen that a 12.5 mg./Kg. dose of sulfadimethoxine produced a peak level of 6.2 mg./100 ml. at two hours, followed by a fairly constant low therapeutic level during the first 24 hours, and only trace levels were present thereafter. Reference to figure 3 indicates that the levels obtained with sulfadimethoxine with this dosage were slightly greater during the first 24 hours than those obtained with either sulfamethoxypyridazine or its acetyl derivative. This disparity was not regarded as significant, however.

When a single 25 mg./Kg. dose of sulfadimethoxine was administered, a peak level of 8.5 mg./100 ml. was achieved within four hours (table I); thereafter, the level declined rather slowly and was still present in a therapeutically significant concentration at the end of 24 hours. At the end of 48 hours, the drug was present in suboptimal amounts and in trace quantities after 72 hours. Comparison between the three compounds (fig. 4) reveals very close correspondence between the blood levels achieved with this dosage of sulfadimethoxine and sulfamethoxypyridazine throughout the entire 72 hour period; however, N'-acetyl sulfamethoxypyridazine produced significantly lower levels than either of the other two drugs with a single dose of 25 mg./Kg.

When a single 50 mg./Kg. dose was administered, the average blood level, after two hours, was 13.4 mg./100 ml., followed by a slowly increasing concentration during the next six hours, with a peak level of 15.4 mg./100 ml. achieved at eight hours

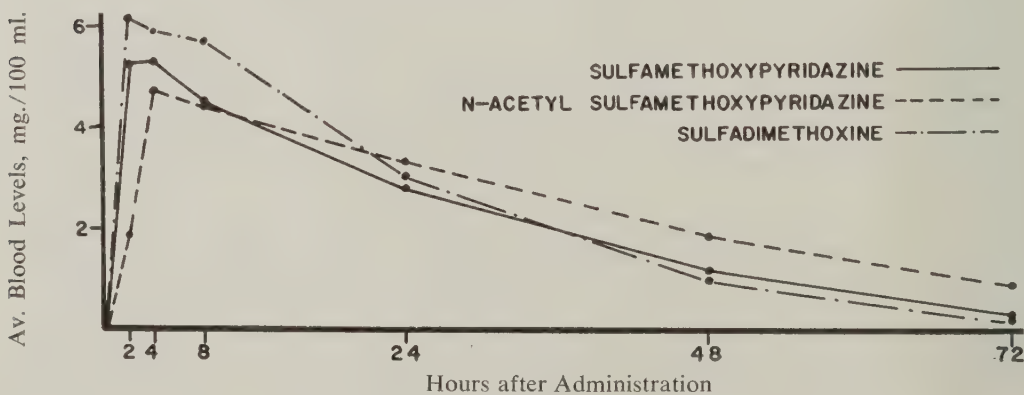


FIG. 3. Comparison of sulfonamide blood levels of three sulfonamides after oral administration of a single dose, 12.5 mg./Kg.

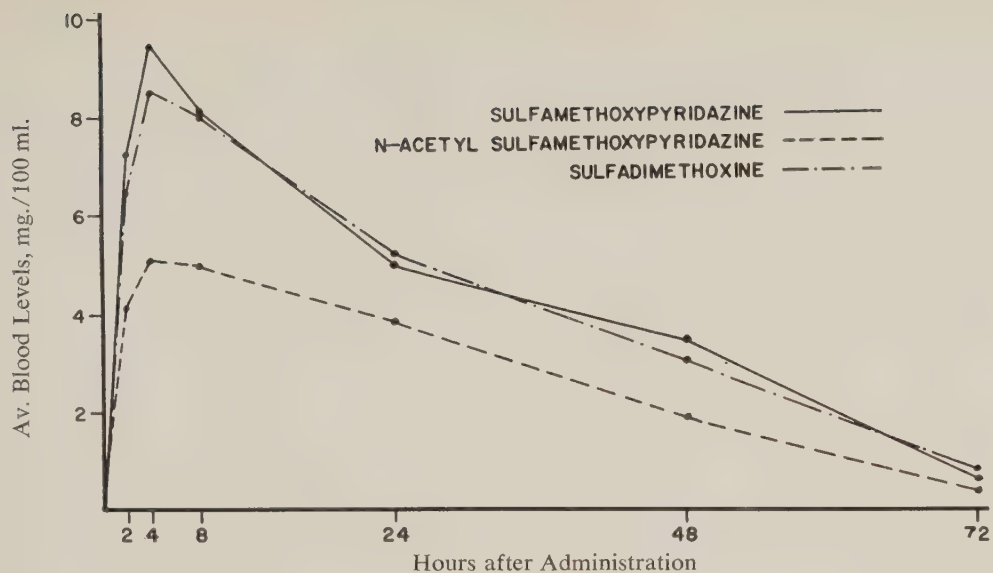


FIG. 4. Comparison of sulfonamide blood levels of three sulfonamides after oral administration of a single dose, 25 mg./Kg.

(table I). Thereafter the level tapered off slowly, with therapeutic concentrations still demonstrable after 48 hours. Reference to figure 5 again depicts the similarity between blood levels attained with sulfadimethoxine and sulfamethoxypyridazine during the entire 72 hour hiatus. Again, N'-acetyl sulfamethoxypyridazine yielded substantially lower levels.

*Blood Levels of Sulfadimethoxine After Repeated Oral Doses.* Simulating the clinical use of sulfadimethoxine, a single daily dose of 25 mg./Kg. was given for

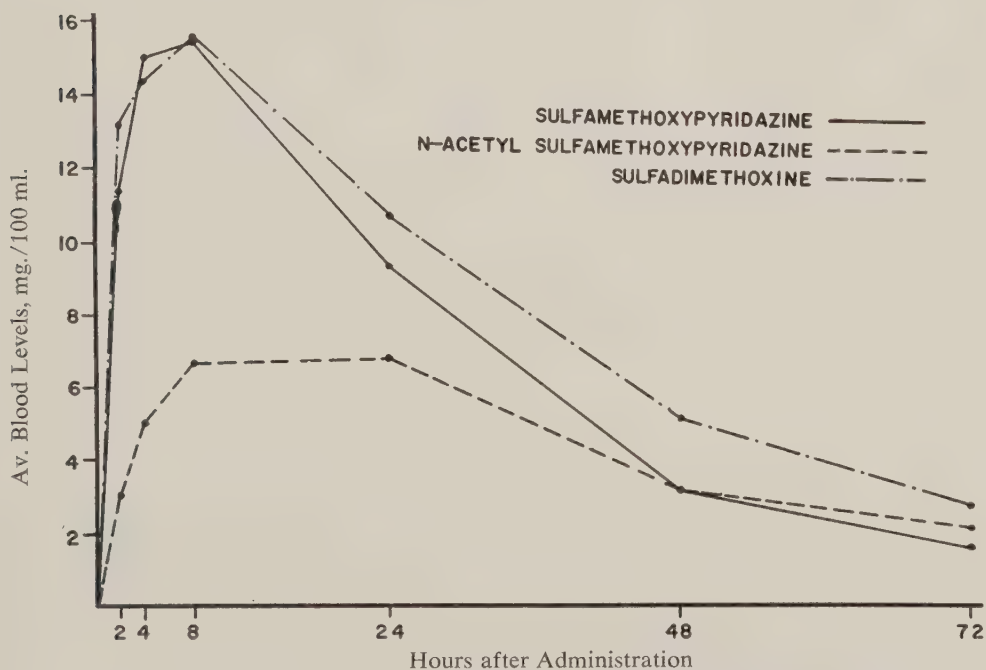


FIG. 5. Comparison of sulfonamide blood levels of three sulfonamides after oral administration of a single dose, 50 mg./Kg.

TABLE II  
Comparison of Blood Levels, mg./100 ml., of 3 Sulfonamides After Repeated  
Oral Dosage of 25 mg./Kg./day

Day	Hour after dose					
	4			24		
	SMP*	Acetyl SMP†	SDM‡	SMP*	Acetyl SMP†	SDM‡
1	8.6	6.5	7.0	7.0	7.2	5.0
2	11.0	12.6	11.6	7.5	7.9	6.2
3	12.9	13.2	11.4	8.8	8.9	6.4
4	14.7	12.9	16.1	8.8	8.0	8.7
5	13.5	Not done	14.6	11.0	Not done	6.6

\* SMP = sulfamethoxypyridazine.

† Acetyl SMP = N'-acetyl sulfamethoxypyridazine.

‡ SDM = sulfadimethoxine.

five consecutive days to 5 children. Free blood sulfonamide levels were determined at 4 and 24 hours after each daily dose, in an attempt to find the highest and lowest blood sulfonamide levels to be expected in therapy. By way of comparison, the same study was carried out with sulfamethoxypyridazine and N'-acetyl sulfamethoxypyridazine, employing an equivalent dosage given to the same number of children. The resulting average blood levels, in mg./100 ml., are presented in table II and figures 6 to 8.

As seen in figure 6, the sulfonamide levels with sulfadimethoxine rose after the first 24 hours and achieved a plateau during the remaining four day period, with levels ranging between 5 and 16.1 mg./100 ml. Figures 7 and 8 depict the levels achieved with N'-acetyl sulfamethoxypyridazine and sulfamethoxypyridazine, respectively; it will be observed that all three of these compounds produced similar maintenance blood levels after the initial 24 hours with this dosage (table II). This would be particularly pertinent in the case of N'-acetyl sulfamethoxypyridazine, since after a single dose the levels were significantly lower than those obtained with the other two drugs; however, with repeated daily doses, the levels for all three preparations paralleled one another closely after the first 24 hours.

*Degree of Conjugation of Sulfadimethoxine in Blood.* The proportion of sulfadimethoxine circulating in the conjugated form varied among individual children but was found to range from 4 to 12 per cent during the first eight hours and from 15 to 25 per cent thereafter. This compares closely to the results reported with sulfamethoxypyridazine, viz., 5 per cent acetylation within the first two to four hours, increasing to 10 to 20 per cent thereafter.

*Degree of Diffusion Across the Blood-Brain Barrier.* Three children with normal meninges, who were receiving sulfadimethoxine, had simultaneous blood and spinal

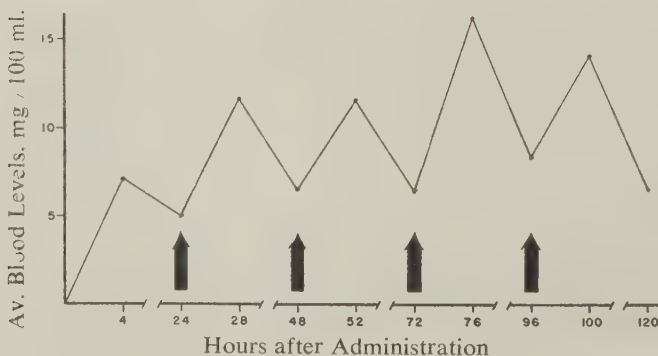
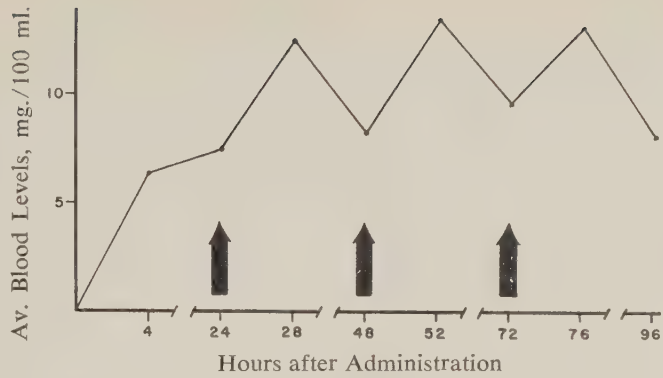


FIG. 6. Sulfadimethoxine blood levels obtained 4 and 24 hours after a daily dose, 25 mg./Kg., given for five consecutive days.

FIG. 7. N'-acetyl sulfamethoxy-pyridazine blood levels obtained 4 and 24 hours after a daily dose, 25 mg./Kg., given for four consecutive days.



fluids assayed for sulfonamide content on the second day of therapy. It was found that only 7.3 per cent of the co-existing blood sulfonamide level was demonstrable in the spinal fluid. In a previous study in children with normal meninges, we reported the diffusion of sulfamethoxypyridazine across the hematocephalic barrier to be only 5.5 per cent.<sup>3</sup> Thus, both drugs diffuse rather poorly and in the same order of magnitude. One would expect some increase in diffusion in instances of inflamed meninges (as has been shown with other sulfonamides), but this point remains to be determined.

INCIDENCE OF SIDE REACTIONS

A total of 63 infants and children received sulfadimethoxine in the present study. Forty-three of these cases were treated in the hospital, where an adequate opportunity presented itself of studying these patients carefully with repeat hemograms, urinalyses, and close observation for untoward clinical manifestations, such as rash, fever, and gastrointestinal disturbances. Generally, treatment consisted of a single sulfadimethoxine daily dose of 25 mg./Kg. for five to eight days. This dose was chosen because of the satisfactory blood levels that could be maintained on such a regimen.

Of the 43 children who were closely observed for untoward reactions, only 1 child developed any symptoms that could be attributed to the drug, consisting of abdominal pain and vomiting on the third day of therapy. No abnormalities of the urine, hemogram, or blood urea nitrogen were observed in any case.

Admittedly, it is much too early to make any categorical statements regarding the paucity of side reactions with sulfadimethoxine. Past experience dictates that extreme caution is indicated in assessing the toxic potential of any new drug (and particularly the sulfonamides), until a substantial number of cases have been treated.

FIG. 8. Sulfamethoxypyridazine blood levels obtained 4 and 24 hours after a daily dose, 25 mg./Kg., given for five consecutive days.

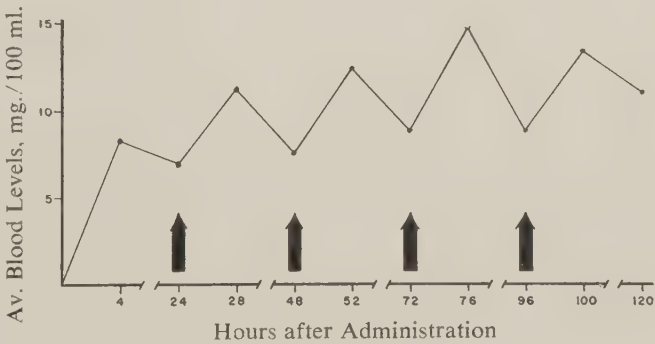


TABLE III  
Clinical Evaluation of Sulfadimethoxine in Pediatric Patients

Infection	Result		
	Good	Fair	Poor
Upper respiratory infections: pharyngitis, tonsillitis, otitis	17	2	
Bronchopneumonia	2		
<i>Shigella</i> dysentery	5		
<i>Salmonella</i> enteritis (group B)			1
Total	24	2	1

Our early experience with sulfamethoxypyridazine was similarly characterized by an almost complete absence of side reactions.<sup>3</sup> However, subsequent experience with this drug has revealed that an occasional child will show reactions, such as rash, nausea, vomiting, headache, fever, and bone marrow depression. In rare instances, these reactions may be dangerously severe. A case of erythema multiforme bullosum (Stevens-Johnson syndrome) was seen at Children's Hospital, following the administration of sulfamethoxypyridazine in a dosage of 17 mg./Kg./day for 10 days. Recently, another case of Stevens-Johnson syndrome, following the administration of N'-acetyl sulfamethoxypyridazine, has come to our attention in the Washington area.<sup>4</sup>

In view of the similarity in structural formula between sulfamethoxypyridazine and sulfadimethoxine, one should be alert to the possibility of untoward reactions as the latter drug is used on a more widespread scale.

#### CLINICAL EVALUATION

Up to the present writing, a group of 27 children, ranging in age from 18 months to 13 years old, has been treated with sulfadimethoxine in a 25 mg./Kg. daily dosage for a variety of bacterial infections. The duration of therapy varied from 7 to 12 days. The diseases treated, with the clinical and laboratory response designated as good, fair, or poor, are presented in table III. The response was considered to be good in 24 or 89 per cent of the patients.

For comparative purposes, a group of 34 children of comparable age were treated with sulfamethoxypyridazine (table IV). With a few exceptions, the type and severity of bacterial infections in both groups were, to a first approximation, similar. As will be noted, the response in the group that received sulfamethoxypyridazine was good in 28 or 82 per cent of the patients. Thus, the clinical response to both of these long-acting sulfonamides appeared to approximate one another closely.

TABLE IV  
Clinical Evaluation of Sulfamethoxypyridazine in Pediatric Patients

Infection	Result		
	Good	Fair	Poor
Upper respiratory infections: pharyngitis, tonsillitis, otitis	12		3
Bronchitis	4		1
Bronchopneumonia	6		2
<i>Shigella</i> dysentery	2		
Cystitis	2		
Cellulitis	2		
Total	28	0	6

1. The object of the present study was to obtain a preliminary evaluation of sulfadimethoxine, a new, long-acting, antibacterial sulfonamide in infants and children.

2. In calibrating the optimal dosage of sulfadimethoxine, it was found that a single daily dose of 25 mg./Kg. produced adequate free blood sulfonamide levels for therapy of the moderate to severe sulfonamide-susceptible bacterial infection. In mild infections, an initial loading dose of 25 mg./Kg., followed by a daily dose of 12.5 mg./Kg., would probably suffice. A similar dosage recommendation would apply for sulfamethoxypyridazine and N'-acetyl sulfamethoxypyridazine.

3. The proportion of sulfadimethoxine circulating in the conjugated form varied among individual children but was found to range from 4 to 12 per cent during the first eight hours and from 15 to 25 per cent thereafter. This compares closely to results reported with sulfamethoxypyridazine.

4. In children with normal meninges, there was relatively little diffusion (7.3 per cent) of sulfadimethoxine across the blood-brain barrier into the spinal fluid. This compared closely with the diffusion (5.5 per cent) of sulfamethoxypyridazine.

5. A group of 63 infants and children was observed for any evidence of untoward reactions after the administration of sulfadimethoxine. Of this group, only 1 child developed any symptoms that could be attributed to the drug, consisting of abdominal pain and vomiting on the third day of therapy. No other clinical side reactions were observed, nor were any abnormalities of the urine, hemogram, or blood urea nitrogen seen in any patient. However, caution would dictate that the assessment of the toxic potential of sulfadimethoxine must await the widespread use of the drug in a substantial number of patients.

6. A group of 27 hospitalized infants and children were treated with sulfadimethoxine in a 25 mg./Kg. daily dosage for a variety of bacterial infections, including upper respiratory infections, bronchopneumonia, *Shigella* dysentery, and *Salmonella* enteritis. The duration of therapy varied from 7 to 12 days. The response to therapy was good in 24 or 89 per cent of the patients. These results closely approximated those observed with sulfamethoxypyridazine in a comparable series of cases.

#### ACKNOWLEDGMENTS

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We are indebted to Mr. John Hercules for his technical assistance.

#### BIBLIOGRAPHY

1. BRATTON, A. C., AND MARSHALL, E. K., JR.: A new coupling component for sulfanilamide determination, *J. Biol. Chem.* 128:537, 1939.
2. JONES, W. F., AND FINLAND, M.: Sulfamethoxypyridazine and sulfachloropyridazine, *Ann. New York Acad. Sc.* 69:473, 1957.
3. ROSS, S.; AHRENS, W. E., AND ZAREMBA, E. A.: Sulfamethoxypyridazine, a long-acting sulfonamide; some preliminary clinical and laboratory observations in infants and children, *Ann. New York Acad. Sc.* 69:501, 1957.
4. SCANLON, R.: Personal communication.

# Preliminary Report of Clinical Experience with Sulfadimethoxine, a New Long-acting Sulfonamide

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The advent of the sulfonamides ushered in a new era in antimicrobial therapy. These chemotherapeutic agents have been used successfully for more than 20 years for a variety of systemic infections.<sup>1-3</sup> However, the hazard of certain side effects, involving especially the urinary tract,<sup>4</sup> has led to the search for new, effective compounds that would eliminate or minimize these reactions.<sup>5-9</sup> One aspect of this objective in chemotherapeutic research is the development of a long-acting compound to produce sustained therapeutic serum levels after a single dose. A new sulfonamide, sulfadimethoxine (Madribon\*), has been employed in the treatment of pediatric patients with infections of the ear, upper respiratory tract, and gastrointestinal system.

The purpose of this study was twofold: (1) to determine the dosage of sulfadimethoxine necessary to produce a therapeutic serum level and that required to maintain this level if administered at 24 hour intervals, and (2) to test clinical effectiveness and safety of this sulfonamide in pediatric patients with various bacterial infections.

*Sulfadimethoxine Serum Level Determinations.* The serum sulfonamide levels were determined by the micromethod of Bratton and Marshall,<sup>10</sup> modified and standardized by Rosenthal of the Rochester General Hospital. The determinations were done against crystalline sulfadimethoxine as the standard. A value greater than 7 mg./100 ml., according to the work of Brickhouse et al,<sup>11</sup> Rhoads et al,<sup>12</sup> and Loughlin and Mullin,<sup>13</sup> was taken as the criterion of adequate or therapeutic level.

A single dose of 30 mg./Kg. body weight of sulfadimethoxine was given to patients who were admitted to the pediatric service for correction of noninfectious surgical conditions, such as inguinal hernias. Sulfadimethoxine levels were determined 4, 8, 12, 24, and 48 hours after the medication had been given. Deterioration curves of sulfadimethoxine were thus obtained (fig. 1).

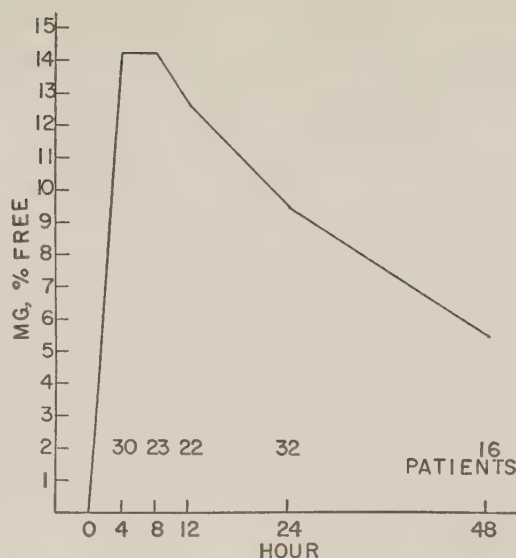
It soon became apparent that in infants less than 1 year old the serum levels were consistently higher with this dose, as illustrated by the following case: When given 30 mg./Kg., a female infant weighing 3.6 Kg. showed serum levels of 30.7 mg./100 ml. in four hours, 25.8 mg./100 ml. in eight hours, 24.0 mg./100 ml. in 12 hours, 18.4 mg./100 ml. in 24 hours, and 5.7 mg./100 ml. in 48 hours. When the dosage of sulfadimethoxine was decreased to 10 mg./Kg., the levels were: 6.9 mg./100 ml. in four hours, 5.6 mg./100 ml. in eight hours, 4.7 mg./100 ml. in 12 hours, 3.0 mg./100 ml. in 24 hours, and 1.2 mg./100 ml. in 48 hours. Since these are probably below therapeutic levels, a schedule of 20 mg./Kg. of body weight was adopted as the initial dosage for all infants less than 1 year of age, and 30 mg./Kg. for children older than 1 year.

Since deterioration curves showed a very slow rate of fall, a maintenance dosage of 15 mg./Kg. in older children and 10 mg./Kg. in infants less than 1 year was adopted. In 26 patients thus treated, reliable steady blood levels were maintained

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\* The trade name of Hoffmann-La Roche, Inc., for sulfadimethoxine is Madribon.

FIG. 1. Deterioration curve of sulfadimethoxine as measured in the number of patients indicated. A single dose of 30 mg./Kg. was administered.



by 30 mg./Kg. as the initial and 15 mg./Kg. as the maintenance dosage. Figure 2 demonstrates the consistent blood levels obtained in 9 patients over a prolonged period of time by the use of the dosage schedules just mentioned.

*Sulfadimethoxine Therapy in Bacterial Infections.* MATERIALS AND METHODS. The study comprised 167 infants and children with presumed or proved bacterial infections. Of these, 130 were seen in private practice and 37 were under treatment by the pediatric service of the Rochester General Hospital. Since all were pediatric patients, the disease categories are those commonly encountered in pediatric practice, i.e., otitis media, bronchitis and other respiratory infections, as well as some instances of gastroenteritis. Table I shows the composition of the group with reference to the diagnostic conditions treated.

The patients treated at the Rochester General Hospital were routinely subjected to the following tests: nasopharyngeal cultures, complete blood counts, and determinations of the serum sulfadimethoxine levels. The nasopharyngeal cultures showed predominantly: pneumococcus, hemolytic *Staphylococcus aureus*, *Staphylococcus pyogenes* both resistant and sensitive strains, coliform bacilli, and *Hemophilus influenzae*.

Of the 37 patients in the pediatric service of the Rochester General Hospital, 17 were followed in the pediatric clinic and 20 were treated as inpatients on the pe-

FIG. 2. Serum levels of sulfadimethoxine taken at random in 9 patients over a five day period. Each dot indicates one sample. The constancy of the blood level is demonstrated. Initial dose was 30 mg./Kg., followed by 15 mg./Kg. once daily thereafter.

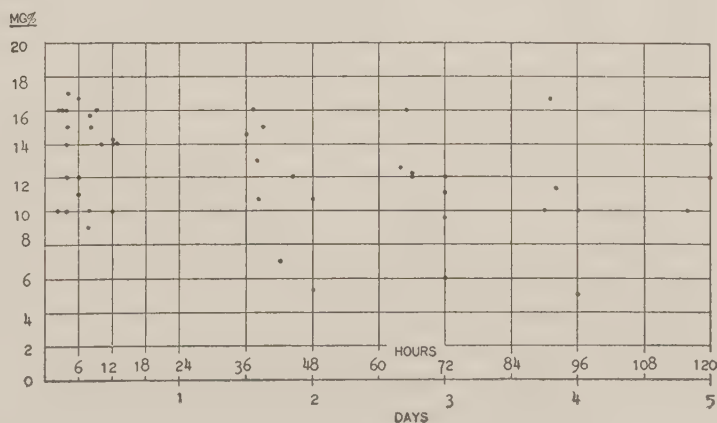


TABLE I  
Results of Sulfadimethoxine Therapy

Disease	No. of patients	Success	Failure
<i>130 Patients Treated in Private Practice</i>			
Otitis media	66	60	6
Bronchopneumonia	6	6	—
Bronchitis	11	10	1
Obstructive laryngotracheitis	3	3	—
Purulent tonsillitis	14	14	—
Cervical adenitis	13	13	—
Purulent rhinitis or sinusitis	8	7	1
Gastroenteritis	7	2	5
Cystitis	2	2	—
Total	130	117	13
Per cent		90	10
<i>37 Patients Treated at the Rochester General Hospital</i>			
Otitis media	6	5	1
Purulent rhinitis	11	9	2
Tonsillitis	7	4	3
Osteomyelitis with penicillin therapy	1	1	—
Ulcerative colitis	1	1	—
Lobar and bronchopneumonia with penicillin therapy	7	7	—
Lobar and bronchopneumonia failing to respond to penicillin therapy	2	2	—
Bronchopneumonia	1	1	—
Gastroenteritis	1	1	—
Total	37	31	6
Per cent		84	16

diatric ward. All were under treatment with sulfadimethoxine for 7 to 10 days according to the clinical indications.

RESULTS. Sulfadimethoxine was effective in 90 per cent of the private patients and 84 per cent of those treated at the Rochester General Hospital. Of the 167 patients, 18 (13 in private practice and 5 on the pediatric service) did not respond to this chemotherapeutic agent. These patients were in the following clinical categories: 7, otitis media; 5, gastroenteritis; 3, bronchitis; 3, tonsillitis. In one case of a 5 year old white boy treated for recurrent otitis media, the drug was not effective, and finally a unilateral mastoidectomy was performed. The unoperated side continued to flare up periodically uncontrolled by sulfadimethoxine therapy, despite the fact that the serum levels were adequate (18 mg./100 ml.). In 4 of the failures in the gastroenteritis group the cultures showed a mixed flora. Two patients with otitis media who failed to respond had hemolytic *Staphylococcus aureus* in the ear culture.

No side reactions to sulfadimethoxine were observed in the entire series of 167 patients.

Of the 10 patients with pneumonia, 3 are of special interest.

*Case 1.* A 17 month old white boy with a lobar pneumonia of the left upper lobe and bilateral bronchopneumonia of the lower lobes was admitted to the Rochester General Hospital. On admission the rectal temperature was 104.4 F. and the white blood count was 41,000/cu. mm., with an increase in the immature forms of the polymorphonuclear leukocytes. Pharyngeal culture showed a predominance of coliform bacilli. A dose of 300 mg. of sulfadimethoxine was given initially, followed by 150 mg. once daily for seven days thereafter. The clinical condition of the child improved rapidly, the temperature returned to normal, and the white blood count fell to 9000/cu. mm. by the third day of hospitalization. The serum sulfadimethoxine levels were consistently greater than 10 mg./100 ml. throughout the course of treatment.

*Case 2.* A 4 year old white girl was admitted with a bilateral pneumonia. The white blood count was 30,700/cu. mm. with an increase of the immature forms of the segmented neutrophils. Hemolytic *Staph. aureus* was recovered in almost pure culture from nose and throat. The patient had been treated with penicillin for four days prior to admission, and this was continued for three days after hospitalization. After an apparent transient response, the temperature rose again and there was no clinical improvement. Sulfadimethoxine was then begun and the temperature returned to normal within 36 hours. Serum sulfonamide levels averaged 13 mg./100 ml. The clinical condition of the child also improved, and she was discharged on the seventh hospital day.

*Case 3.* A 13 year old white girl was admitted with a lobar pneumonia in the right upper lobe and bronchopneumonic infiltrations in the left lower lobe. Nasal and pharyngeal cultures showed streptococci and coliform bacilli. The child was treated with penicillin for seven days with no clinical response. The temperature curve showed daily spikes up to 101 and 102 F. Roentgenograms showed increase in the pneumonic infiltrations. On the seventh day penicillin was discontinued and 1250 mg. of sulfadimethoxine was given as the initial dose, followed by 625 mg. daily. On the third day of treatment, the temperature returned to normal. Eight days later the child was discharged, afebrile. There was marked improvement in the roentgenographic changes noted in the lung fields. Serum sulfonamide levels averaged 16 mg./100 ml. throughout the course of therapy.

The following case illustrates the result of treatment with sulfadimethoxine in an infection of the urinary tract.

*Case 4.* A 19 month old white girl was admitted with pyelitis. *Escherichia coli* in pure culture were obtained from the cultured urine. The child received 400 mg. sulfadimethoxine as an initial dose and 200 mg./day afterward. The temperature began to decrease on the second day of this therapy, and four days later, the urine culture, obtained in the operating room during cystoscopy, was sterile.

The following case illustrates the result of treatment with sulfadimethoxine in gastroenteritis.

*Case 5.* A 4 year old white girl was admitted with gastroenteritis. The stool culture showed the presence of a *Salmonella*-type organism. After treatment for four days the stool culture was negative for this organism and the patient's clinical condition had improved.

#### DISCUSSION

In most instances no specific cause for failure of therapy could be found. In the case of the boy with the recurrent otitis media, failure may have been due to the fact that sulfonamides characteristically are not so effective against bacteria in the presence of pus as in its absence. Failure in the 2 patients whose cultures showed the presence of hemolytic *Staph. aureus* may signify resistance to this chemotherapeutic agent. In 4 of the failures in the gastroenteritis group, the causative agent in the mixed flora was presumed to be of viral origin, in a season of the year when viral infections were prevalent.

The success with the use of sulfadimethoxine is comparable to or better than that attained by other sulfonamides. Continued experience with this drug since submission of this paper indicates that these figures can be applied to a larger series. Thus, sulfadimethoxine by single doses daily is an effective chemotherapeutic agent maintaining active blood levels and thus far showing no side reactions.

It is of interest that though the drug failed to relieve the infection in 2 cases presumed due to hemolytic *Staph. aureus*, it has been used since submission of the original statistics in 6 cases of generalized pustular dermatitis from which hemolytic *Staph. aureus* was isolated. Two of these proved to be of the resistant strain (type 80/81) in newborn infants from a nursery where this organism is known to exist.

1. Sulfadimethoxine is capable of maintaining effective serum sulfonamide levels with only one daily oral dose.
2. Sulfadimethoxine is effective in most common infections in the pediatric age group.
3. In older children, daily dosages of 15 mg./Kg. body weight were sufficient to maintain these levels after an initial dose of 30 mg./Kg. In infants less than 1 year, the respective dosages are 10 mg./Kg. for maintenance and 20 mg./Kg. initially.
4. The failure of this treatment in 10 per cent of the cases may possibly be explained by the fact that this study was initiated in a season when viral infections are prevalent.
5. The combination of antimicrobial effectiveness plus the lack of undesirable side effects make sulfadimethoxine an essentially useful drug.

## BIBLIOGRAPHY

1. KOLMER, J. A.: Progress in chemotherapy of bacterial and other diseases, with special reference to the prontosils, sulfanilamide and sulfapyridine, *Arch. Int. Med.* 65:671, 1940.
2. GOLDSTEIN, A.: Antibacterial chemotherapy, *New England J. Med.* 240:98, 1949.
3. HAWKINS, F., AND LAWRENCE, J. S.: *The Sulfonamides*, New York, Grune & Stratton, Inc., 1951.
4. CARROLL, G.: Current use of sulfonamides in urology, *Ann. New York Acad. Sc.* 69:395, 1957.
5. PRICE, P. C., AND HANSEN, A. E.: Blood levels in relationship to dosage of 3,4-dimethyl-5-sulfanilamido isoxazole (Gantrisin) in children with clinical observations, *Texas Rep. Biol. & Med.* 9:764, 1951.
6. BUSH, W. L.: The treatment of non-specific urinary infections, *South. M. J.* 45:871, 1952.
7. YOW, E. M.: Observations on the use of sulfoxazole (Gantrisin) in 1,000 consecutive patients with particular reference to the frequency of undesirable side effects, *Am. Pract. & Digest Treat.* 4:521, 1953.
8. RHOADS, P. S.; BILLINGS, C. E., AND O'CONNER, V. J.: Anti-bacterial management of urinary tract infections, *J.A.M.A.* 148:165, 1952.
9. McMARTIN, W. J., AND MILAM, D. F.: Recent trends in urologic practice, *J.A.M.A.* 148:1117, 1952.
10. BRATTON, A. C., AND MARSHALL, E. K., JR.: A new coupling component for sulfanilamide determination, *J. Biol. Chem.* 128:537, 1939.
11. BRICKHOUSE, R. L.; LEPPER, M. H.; STONE, T. E., AND DOWLING, H. F.: The treatment of pneumonia and other infections with a soluble sulfonamide, Gantrisin (NU-445; 3,4-dimethyl-5-sulfanilamido-isoxazole), *Am. J. M. Sc.* 218:133, 1949.
12. RHOADS, P. S.; SVEC, F. A., AND ROHR, J. A.: Bacterial meningitis, *A.M.A. Arch. Int. Med.* 85:259, 1950.
13. LOUGHLIN, E. H., AND MULLIN, W. G.: The relative absorption and elimination of sulfoxazole and acetyl sulfoxazole, *Antib. & Chemo.* 5:609, 1955.

# Blood and Cerebrospinal Fluid Levels of Sulfamethoxypyridazine after Intravenous Administration in Children

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In previous studies we<sup>1</sup> and others<sup>2,3</sup> have shown sulfamethoxypyridazine, in oral preparation, to be an effective and safe therapeutic agent in children. Recently, sulfamethoxypyridazine\* was released in an intravenous preparation for clinical trials. Previous investigations<sup>4</sup> with the oral preparation showed that sulfamethoxypyridazine diffused readily into the spinal fluid of normal persons and was used successfully in the treatment of meningococcus meningitis.<sup>5</sup>

With sulfamethoxypyridazine, good therapeutic blood levels are attained with a relatively lower dosage schedule than with the more commonly used sulfonamides. Its other properties of reduced renal complications<sup>6</sup> and clinical toxicity suggested that intravenous sulfamethoxypyridazine would be useful in the treatment of meningitis, where rapidly high blood levels and low toxicity are essential in effective treatment. Because of the afore-mentioned factors, it was thought advantageous to investigate this new intravenous preparation of sulfamethoxypyridazine in children.

## BLOOD LEVELS

*Materials and Methods.* The stock sulfamethoxypyridazine (12.5 or 25 per cent) intravenous preparation was diluted to a 5 per cent preparation with normal saline before injection. The 5 per cent dilution was slowly injected intravenously during a period of two to four minutes. A new 10 or 2 ml. ampoule of sulfamethoxypyridazine was used for each injection, and once the ampoule was open and/or diluted to a 5 per cent solution, it was immediately discarded after the injection was given.

In the first part of the study, single loading doses of sulfamethoxypyridazine were injected intravenously into children of different weights, and whole blood levels were determined at  $\frac{1}{4}$ ,  $\frac{1}{2}$ , 1, 2, 4, 8, 12, 24, and 48 hours. Blood level determinations were performed by a microtechnique using blood obtained by skin puncture and analyzed by the Bratton-Marshall<sup>7</sup> method with a standardization curve determined from a sample of pure sulfamethoxypyridazine. From these data a loading dose of 40 to 50 mg./Kg. was chosen.

Next, a maintenance dosage was determined by giving a loading dose with a maintenance dose every 12 hours and doing blood levels of sulfamethoxypyridazine immediately before the maintenance dose was given and 30 minutes afterward. From these data a maintenance dosage of 20 to 25 mg./Kg. was chosen.

Blood counts and urine examinations were done periodically on all the children while they were receiving the drug.

*Results.* Data concerning blood levels of sulfamethoxypyridazine (fig. 1, table I) suggest that the drug would persist at high levels over a long period of time, as was previously noted for the oral preparation. Peak levels occurred at approximately 15

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Aided by a grant from Lederle Laboratories Division, American Cyanamid Co.

\* The trade name of Lederle Laboratories Division, American Cyanamid Co., for intravenous sulfamethoxypyridazine is Lederkyn. This drug was supplied for the study by this firm.

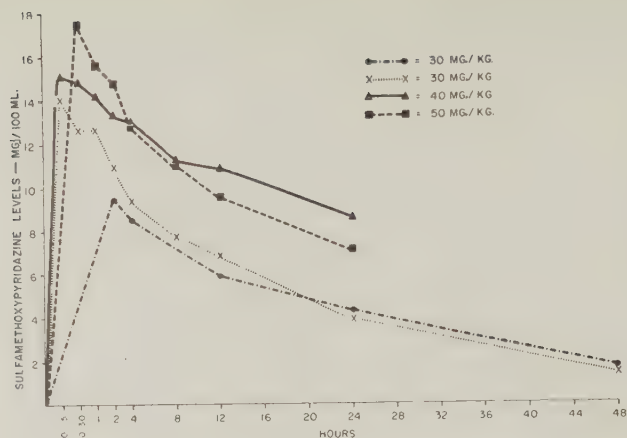


FIG. 1. Levels of sulfamethoxypyridazine in whole blood following the administration of single doses of the drug are indicated.

to 30 minutes after injection, and satisfactory levels were recorded at 24 hours. At 48 hours a significant amount of sulfamethoxypyridazine was still present in the blood.

Because we felt that the intravenous sulfamethoxypyridazine would be valuable in the treatment of meningitis, where high blood levels are required, we chose to give the maintenance dose every 12 hours so that the blood level of sulfamethoxypyridazine would be sure to remain in the therapeutic range.

Data concerning the loading dose plus a maintenance dose every 12 hours of 20 to 25 mg./Kg. are shown in figure 2 and table II. Good therapeutic blood levels were maintained with these dosages, but there is a tendency to get accumulation with increasing levels on about the fourth day. The blood level for patient 19 at 60 hours was not figured in the calculations because it was assumed to be a technical error.

#### SPINAL FLUID LEVELS

*Methods.* In the first part of the study, 15 infants and children without central nervous system infections were selected, and blood and spinal fluid levels of sulfamethoxypyridazine were done at varying intervals of time. The patients were given a sulfamethoxypyridazine loading dose of 40 to 50 mg./Kg. and a maintenance dose of 20 to 25 mg./Kg. every 12 hours. Spinal fluid levels were done at two or four hours after the loading dose was given. In some cases additional spinal fluid determinations were done at 12 and 24 hours after the loading dose, but in each instance before the maintenance dose was given. In one case (16) a spinal fluid level was done at 60 hours, before the maintenance dose was given.

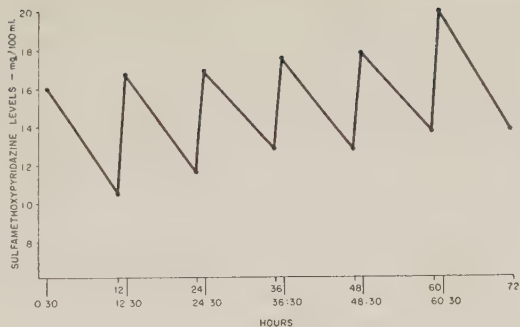
In the second part of this study 4 children with infections of the central nervous system (3 viral meningoencephalitis, 1 *Hemophilus influenzae* meningitis) had spinal fluid level determinations done according to the previously mentioned schedule. In the case of meningitis, sulfamethoxypyridazine was substituted for the usual

TABLE I

*Sulfamethoxypyridazine Levels in Whole Blood after a Single Intravenous Dose*

Pt.	Age	Length, inches	Wt., lb.	Surface Total			Time, hr.										
				area, sq. M.	dosage, mg.	Mg. /Kg.	¼	½	1	2	4	8	12	24	48		
1	18 mo.	33	25.8	0.5	350	30	—	—	—	9.5	8.5	—	5.9	4.2	1.6		
2	7 mo.	26.5	16.8	0.38	225	30	14.1	12.6	12.5	11.0	9.3	7.8	6.9	3.9	1.5		
3	8 mo.	26	15	0.34	275	40	15.1	14.8	14.2	13.4	13	11.2	10.9	8.5	—		
4	2 yr.	34	23.6	0.5	500	50	—	17.5	15.7	14.7	12.9	11	9.6	7.1	—		

FIG 2. Average levels of sulfamethoxypyridazine in whole blood of 4 patients following the intravenous injection of 40 or 50 mg./Kg. initially and 20 or 25 mg./Kg. every 12 hours are given. (See table II.) Blood samples were obtained just prior to and 30 minutes after injection.



sulfonamide used in the combined therapy of meningitis at this hospital. The combined therapy consists of a sulfonamide, penicillin, and chloramphenicol.

*Results.* Table III shows blood and spinal fluid levels of sulfamethoxypyridazine and their time relationship to the injection of the drug. The ratios of spinal fluid concentration to blood concentration are given in table IV. This figure expresses the permeability of the blood-brain barrier to the drug.

The penetration of sulfamethoxypyridazine into the spinal fluid in children without central nervous system disease compared well with similar studies using other sulfonamides.<sup>8,9</sup> The range of permeability into the spinal fluid was 11 to 22.1 per cent of the blood level. However, it is of interest that the infant with the highest permeability (31.6 per cent) showed no evidence clinically of central nervous system disease, but was moribund on admission and was thought to have an overwhelming virus infection. This infant died within 20 hours of admission to the hospital. Because of the infant's overwhelming infection, we hesitate to consider him as not having central nervous system infection, even though the spinal fluid was considered to be normal.

In general, the spinal fluid determinations done at four hours after the loading dose tended to be slightly higher than those done at two hours. Determinations done 12 hours after either the loading or the maintenance dose were in the same range as the two and four hour determinations, which would indicate that the drug is well maintained once it gets into the spinal fluid. The children with central nervous system infections showed a slightly higher permeability of the drug into the spinal fluid. The range of permeability into the spinal fluid was 15.9 to 28.1 per cent of the blood level.

*Discussion.* It is felt that the data presented here indicate that high therapeutic whole blood levels of intravenous sulfamethoxypyridazine may be maintained in children by administration of the drug at 12 hour intervals in dosages less than those

TABLE II  
Sulfamethoxypyridazine Levels in Whole Blood after Intravenous Administration of an Initial Dosage of 40 to 50 mg./Kg. and a Maintenance Dosage of 20 to 25 mg./Kg.

Pt.	Age, yr.	Dosage	Wt., lb.	Time, hr.											
				½	12	12½	24	24½	36	36½	48	48½	60	60½	72
16	9	40/20	60	16	10.7	15.1	12.1	16.5	11	15.6	11.9	15.9	11.4	16	12.6
17	8	40/20	51	13.4	8.9	15.2	11.1	13.9	12.9	16.6	12.6	17.8	15.0	20.3	14.3
18	2	50/25	28	17.5	11.3	17.3	12.1	18.9	13.8	19.4	14.3	19.3	14.8	22.3	14.7
19	11/12	50/25	20.5	17.3	11.2	19.6	11.5	19.0	14.1	19.3	12.5	18.6	32.7*	21.8	14.0
Average (4)				16.0	10.5	16.8	11.7	17.0	12.9	17.7	12.8	17.9	13.7	20.1	13.9
Range Lowest				13.4	8.9	15.1	11.1	13.9	11.0	15.6	11.9	15.9	11.4	16.0	12.6
Highest				17.5	11.3	19.6	12.1	19.0	14.1	19.4	14.3	19.3	15.0	22.3	14.7

\* Not used in calculations.

usually employed with other sulfonamide drugs.<sup>9</sup> From this study, we would recommend a loading dosage of 50 mg./Kg. and a maintenance dosage of 25 mg./Kg. every 12 hours for children 2 years of age or less and a loading dosage of 40 mg./Kg. and a maintenance dosage of 20 mg./Kg. every 12 hours for children more than 2 years of age. On this dosage schedule, in some children, there was a tendency to get increasing blood levels on about the fourth day of intravenous injection of sulfamethoxypyridazine.

This new intravenous form of sulfamethoxypyridazine offers a rapid and safe way of getting high therapeutic blood levels in children who are severely ill or are unable to take the oral preparation.

Intravenous sulfamethoxypyridazine penetrated the blood-brain barrier well in normal children and remained there at good levels between the 12 hour doses of the drug. However, our work does not substantiate the findings of others who re-

TABLE III  
*Whole Blood and Spinal Fluid Level of Sulfamethoxypyridazine after Intravenous Administration of an Initial Dosage of 40 to 50 mg/Kg. and a Maintenance Dosage of 20 to 25 mg./Kg.*

Pt.	Age	Wt., lb.	Dosage	Time, hr.										
				¼	½	2	4	12	12½	24	36	48	60	72
5	18 mo.	24.0	40/20	15.7	14.7	13.0	—	9.9	13.8	11.8	10.0	7.6†	—	—
						1.6°		1.7°		1.65°				
6	7 mo.	15.3	40/20	15.1	13.0	11.1	—	7.5	15.1	9.8	10.7	7.5†	—	—
						1.29°		1.3°		1.34°				
7	2½ yr.	24.0	40/20	11.8	13.1	11.4	—	7.3	11.9	8.6	8.9	—	—	—
						1.26°				1.11°				
8	6 mo.	12.1	40/20	12.8	12.6	10.8	—	6.9	11.9	9.2	6.6†	4.8‡	—	—
						1.42°		1.09°		1.44°				
9	4 mo.	14.1	40/20	10.8	13.8	9.2	—	7.2	9.9	8.1	8.4	6.0†	—	—
						1.47°		1.28°		1.52°				
10	5 mo.	14.1	50/25	15.1	11.9	11.4	—	7.8	18.5	8.5	8.2	9.3	9.3	10.6
						1.9°		1.68°						
11	4 yr.	31.0	50/25	69.5§	19.0	17.4	—	13.0	22.0	12.7	10.2	11.0	11.4	7.2†
						2.84°		1.82°						
12	5 mo.	12.0	40/20	—	—	8.7	—	6.3	—	—	—	—	—	—
						2.75°								
13	3 yr.	21.8	50/25	17.3	—	—	11.9	8.5	—	8.5	—	—	—	—
							2.63°							
14	6 yr.	40.0	50/25	23.7	—	—	18.5	14.6	—	16.2	—	—	—	—
							2.95°							
15	2 yr.	24.1	50/25	15.9	—	—	9.6	7.8	—	—	—	—	—	—
							2.7°							
16	9 yr.	60.0	40/20	—	16.0	—	13.3	10.7	15.1	12.1	11.0	11.9	11.4	12.6
							2.25°						2.85°	
17	8 yr.	51.0	40/20	—	13.4	—	10.9	8.9	15.2	11.1	12.9	12.6	15.0	14.3
							2.33°							
18	2 yr.	28.0	50/25	—	17.5	—	14.2	11.3	17.3	12.1	13.8	14.3	14.8	14.7
							3.1°							
19	11 mo.	20.5	50/25	—	17.3	—	15.2	11.2	19.6	11.5	14.1	12.5	32.7§	14.0
							2.33°							
20	10 mo.	15.11	50/25	—	14.5	—	13.3	11.2	16.5	12.0	15.5	—	—	—
							2.3°							
21	8 mo.	19.6	50/25	—	17.2	—	14.2	10.8	—	—	—	—	—	—
							2.6°							
22	2 yr.	24.10	50/25	—	26.0	—	13.7	—	—	—	—	—	—	—
							2.9°							
23	2 yr.	21.8	50/25	—	16.0	—	13.3	8.3	—	—	—	—	—	—
							2.1°							

\* Indicates spinal fluid levels of sulfamethoxypyridazine.  
† Indicates no sulfamethoxypyridazine given 12 hours previously.  
‡ Indicates no sulfamethoxypyridazine given 24 hours previously.  
§ Assumed to be a technical error.

ported spinal fluid levels up to 50 per cent of the blood level in animal experiments.<sup>10</sup> The permeability levels we observed compare well with spinal fluid levels reported for other sulfonamides.

There is suggestive evidence that the permeability is increased when central nervous system infection is present. This is to be expected from observations with other sulfonamides. However, our cases are too few to be significant, but spinal fluid levels in them tended to be higher when central nervous system infection was present.

The patient with meningitis did well when sulfamethoxypyridazine was substituted for the usual sulfonamide used in the combined treatment of meningitis at this hospital.

TABLE IV  
*Comparative Blood and Spinal Fluid Levels\**

Patient	Age	Weight, lb.	Hours after last dose	Blood	Cerebro-spinal fluid	Cerebro-spinal fluid/blood	Comment
5	18 mo.	24	2	13.0	1.6	0.123	No central nervous system infection
			12	9.9	1.7	0.171	
			12	11.8	1.65	0.131	
6	7 mo.	15.3	2	11.1	1.29	0.116	Normal central nervous system
			12	7.5	1.3	0.173	
			12	9.8	1.34	0.136	
7	2½ yr.	24.0	2	11.4	1.26	0.110	Normal central nervous system
			12	8.6	1.11	0.129	
8	6 mo.	12.10	2	10.8	1.42	0.131	Normal central nervous system
			12	6.9	1.09	0.158	
			12	9.2	1.44	0.156	
9	40 mo.	14.1	2	9.2	1.47	0.159	Normal central nervous system
			12	7.2	1.28	0.177	
			12	8.1	1.52	0.187	
10	5 mo.	14.1	2	11.4	1.9	0.166	Normal central nervous system
			12	7.8	1.68	0.177	
11	4 yr.	31.0	2	17.4	2.84	0.163	Normal central nervous system
			12	13.0	1.82	0.140	
13	3 yr.	21.8	4	11.9	2.63	0.221	Normal central nervous system
17	8 yr.	51.0	4	10.9	2.33	0.213	Normal central nervous system
18	2 yr.	28.0	4	14.2	3.1	0.218	Normal central nervous system
19	11 mo.	20.5	4	15.2	2.33	0.159	Normal central nervous system
21	8 mo.	19.6	4	14.2	2.6	0.183	Normal central nervous system
22	2 yr.	24.1	4	13.7	2.9	0.211	Normal central nervous system
23	2 yr.	21.8	4	13.3	2.1	0.157	Normal central nervous system
12	5 mo.	12.0	2	8.7	2.75	0.316	Died 20 hours after admission to hospital. Clinical—no evidence of central nervous system involvement
15	2 yr.	24.1	4	9.6	2.7	0.281	Meningitis ( <i>H. influenzae</i> )
14	6 yr.	40.0	4	18.5	2.95	0.159	Viral meningoen- cephalitis
16	9 yr.	60.0	4	13.3	2.25	0.169	Viral meningoen- cephalitis
20	10 mo.	15.11	4	13.3	2.3	0.173	Viral meningoen- cephalitis

\* Figures abstracted from table III.

An additional observation to be made in such a study is the incidence of side reactions attributable to the drug. In this series, 1 patient (16) showed a generalized macular rash, which developed on the third day after the drug was started. We attributed the rash to the viral disease process that the patient had at the time, but it is also possible that the rash was caused by the drug. Blood and urine examinations done while the children were receiving the drug revealed no abnormality of significance, despite the fact that levels encountered were often higher than is considered desirable for the other sulfonamides.

#### SUMMARY

1. An intravenous solution of sulfamethoxypyridazine was given to a group of infants and children to establish a dosage that would maintain adequate therapeutic blood levels. From these data, an initial loading dosage, in infants up to 2 years of age, of 50 mg./Kg. was established followed by a maintenance dosage of 25 mg./Kg. every 12 hours. In older children the loading dosage was established of 40 mg./Kg. followed by a maintenance dosage of 20 mg./Kg. every 12 hours.

2. A comparison of the levels of sulfamethoxypyridazine in the blood and spinal fluid two to four hours after injection of the drug showed the level in the spinal fluid to range between 11 and 22.1 per cent of the blood level in those infants and children without central nervous system involvement, and between 15.9 and 28.1 per cent in those with central nervous system disease.

3. One child developed a generalized macular rash while on therapy with sulfamethoxypyridazine, which may have been a reaction to the drug.

#### ADDENDUM

Since this paper was submitted for publication, an additional case of meningococcemia with meningococcus meningitis was treated with intravenous sulfamethoxypyridazine as the only medication used. The patient completely recovered and his clinical course was similar to that seen when the usual sulfonamide therapy is used in this illness.

#### BIBLIOGRAPHY

1. CLEVELAND, W. W.; SMITH, G. F., AND LAWSON, R. B.: A clinical evaluation of sulfamethoxypyridazine in children. *In: Antibiotics Annual*, New York, Medical Encyclopedia, Inc., 1958, pp. 86-94.
2. WEIHL, C.: Sulfamethoxypyridazine: a new sulfonamide for pediatric outpatients, *AM & CT* 5:173, 1958.
3. RUIZ SÁNCHEZ, F.; RUIZ SÁNCHEZ, A.; ACEVES, R., AND NARANJO GRANDA, E.: The use of sulfamethoxypyridazine (Kynex) in the treatment of infectious diseases, *AM & CT* 5:437, 1958.
4. BOGER, W. P.; STRICKLAND, C. S., AND GYLFE, J. M.: Sulfamethoxypyridazine (Kynex) a new long-acting sulfonamide, *AM & CT* 3:378, 1956.
5. VELASCO-JOVEN, E. A., AND DOMINGO, P.: Meningococcic meningitis treated with sulfamethoxypyridazine (Kynex), *J. Philippine M. A.* 33:584, 1957.
6. VINNICOMBE, J.: Assessment of toxicity of a new sulfonamide, sulfamethoxypyridazine (Kynex), *AM & CT* 5:474, 1958.
7. BRATTON, A. C., AND MARSHALL, E. K.: A new coupling method for sulfanilamide determination, *J. Biol. Chem.* 128:537-550, 1939.
8. BECK, J. M., AND BRAUDE, A. I.: The superiority of a triple sulfonamide preparation in producing high spinal fluid concentrations in meningitis, *AM & CT* 3:454, 1956.
9. DAESCHNER, C. W.; CLARK, J. L., AND YOW, E. M.: A comparative evaluation of sulfonamides, *J. Pediat.* 50:531, 1957.
10. LITCHFIELD, J. R., JR.: Properties of a new antibacterial sulfonamide, Kynex, 3-sulfanilamido-6-methoxypyridazine, *Proceedings XX International Physiological Congress*, Brussels, Belgium, July 30-Aug. 4, 1956.

# Vasomotor Effects Produced by the Prolonged Oral Administration of Antimicrobial Agents in Primary Hypertension, with Special Reference to the Nitrofurans

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After the introduction of antimicrobial agents, numerous reports appeared suggesting that these agents may also have many nonchemotherapeutic effects. The growth-stimulating effect on swine, poultry, dairy calves as well as infants and children has been demonstrated by a number of workers.<sup>1-6</sup> That antibiotics are not inert pharmacologically was recently demonstrated when respiratory arrest was produced by the intraperitoneal injection of neomycin.<sup>7,8</sup> On the other hand, metabolic changes have been produced by the oral administration of chlortetracycline. These consisted of an increase in the urinary excretion of nitrogen, riboflavin, and the "essential" amino acids: tryptophan, histidine and threonine, whereas there was no consistent change in the number and variety of intestinal organisms during or after treatment.<sup>9</sup>

This is a report on the antihypertensive action of two members of the nitrofurans series of antimicrobial compounds (NF-180 and NF-260).

None of the nitrofurans had been found to have any effect on the cardiovascular or respiratory systems until Calesnick and DiPalma<sup>10</sup> first reported the hypotensive action of furazolidone (NF-180)\* while investigating the human pharmacology of this drug.

Furazolidone is bactericidal and highly effective both in vitro and in vivo against a wide variety of microorganisms<sup>11</sup> (table I) by inhibiting microbial enzymatic metabolism.<sup>12</sup> Its structure is shown in figure 1.

Rapid catabolism of nitrofurans occurs by reduction of the 5-nitro group.<sup>13</sup> End products isolated to date have shown no demonstrable antimicrobial activity. Nitrofurans reversibly inhibit the anaerobic formation of acetyl coenzyme A from pyruvate.<sup>14</sup> Aerobically, nitrofurans (specifically nitrofurazone) act as inhibitors in the xanthine oxidase-hypoxanthine system.<sup>15</sup> Continuous intravenous infusion of nitrofurans into normal dogs failed to elicit a vasomotor response.

After peroral administration of furazolidone to normal adults, 0 to 9 per cent appears in the feces and 2 to 8 per cent in the urine, which may be tinted from deep yellow to brown. The blood level is from 0.9 to 3.3 gamma/ml. No drug can be detected chemically in the cerebrospinal fluid, and the blood pressure of normotensive persons is unaltered.

The subjects were studied thoroughly to determine the effectiveness as well as the mode of action of furazolidone in reducing elevated arterial blood pressures. Initially the subjects were carefully screened to evaluate their hypertensive status. The electrocardiograms and eye grounds in many patients revealed changes indicative of elevated diastolic pressure of long duration. After this screening program, 16 subjects, all diagnosed as having uncomplicated primary hypertension, were selected. A majority of them was ambulatory (10), the others (6) being hospitalized. No attempt was made to restrict either salt or caloric intake. These hypertensive patients were observed at weekly intervals at which time all essential data were recorded.

\* The trade name of Eaton Laboratories for furazolidone is Furoxone.

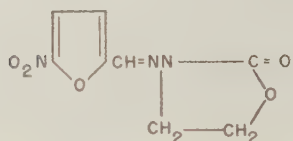
TABLE I

The *in Vitro* Antibacterial Spectrum of Furazolidone as Determined by the Broth Serial Dilution Method

<i>Salmonella paratyphi</i>	<i>Aerobacter aerogenes</i>	<i>Staphylococcus aureus</i>
<i>Salmonella schottmülleri</i>	<i>Klebsiella pneumoniae</i>	<i>Diplococcus pneumoniae</i>
<i>Salmonella typhimurium</i>	<i>Proteus vulgaris</i>	<i>Streptococcus pyogenes</i>
<i>Salmonella enteritidis</i>	<i>Pseudomonas aeruginosa</i>	<i>Streptococcus faecalis</i>
<i>Salmonella typhosa</i>	<i>Vibrio comma</i>	<i>Streptococcus</i>
<i>Salmonella pullorum</i>	<i>Pasteurella pestis</i>	species (anaerobic)
<i>Salmonella gallinarum</i>	<i>Pasteurella bovis</i>	<i>Corynebacterium diphtheriae</i>
<i>Shigella dysenteriae</i>	<i>Brucella abortus</i>	<i>Bacillus subtilis</i>
<i>Shigella dysenteriae</i>	<i>Brucella suis</i>	<i>Bacillus anthracis</i>
<i>Shigella sonnei</i>	<i>Brucella melitensis</i>	<i>Clostridium septicum</i>
<i>Shigella alkaescens</i>	<i>Hemophilus influenzae</i>	<i>Clostridium perfringens</i>
<i>Escherichia coli</i>	<i>Hemophilus pertussis</i>	<i>Clostridium tetani</i>
		Miscellaneous

Preliminary testing in several persons (fig. 2) illustrated clearly the hypotensive effect of furazolidone.

In subsequent studies, all subjects were started on a daily peroral dosage of 800 mg. (200 mg. four times a day), which was reduced gradually (fig. 3) when the hypotensive response became stabilized. Table II shows that in this group, the re-



N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone  
(NF 180)

FIGURE 1.

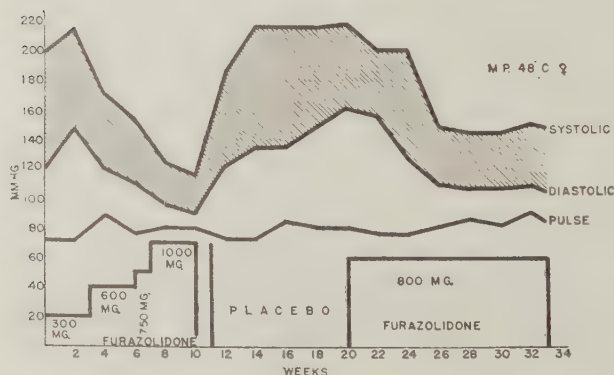


FIG. 2. This patient ran out of medication at the end of the tenth week and returned to the laboratory the following week. She was placed on a placebo, and arterial blood pressure rose to the pre-treatment level. Another course of furazolidone again reduced the blood pressure in six weeks.

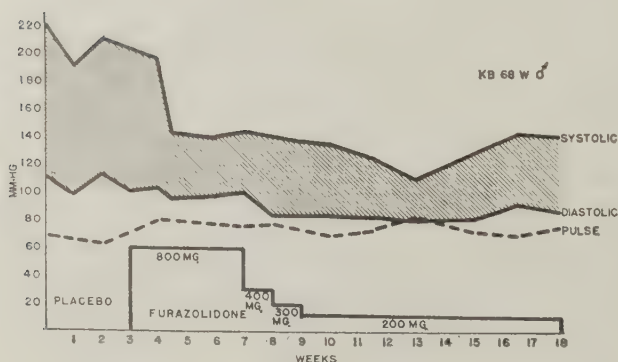


FIG. 3. The reduction in arterial blood pressure was maintained with only one-fourth the initial dose of 800 mg./day. No tolerance observed.

TABLE II

*Effect of Furazolidone in 16 Subjects with Uncomplicated Primary Hypertension*

Pt.	Age, yr.	Race*	Sex	Before treatment			After treatment			
				Blood pressure, mm.Hg (sitting)	Pulse	Weeks re- quired for hypotensive response	Blood pressure, mm.Hg			
							Supine	Sitting	Standing	Pulse
C. B.	59	C	F	192/112	93	5	148/90	134/84	136/86	86
E. B.	65	N	F	198/120	96	4	136/80	120/74	110/68	79
E. B.	48	C	F	204/126	99	3	176/106	162/98	150/104	75
K. B.	68	C	M	202/100	81	2	138/86	136/82	132/92	78
C. C.	68	C	F	220/100	66	8	146/80	148/76	142/78	72
C. F.	45	C	M	180/110	87	4	166/86	160/80	154/78	84
C. F.	42	C	M	226/124	96	2	144/94	140/90	142/92	86
E. F.	62	C	F	216/108	68	2	162/84	160/84	156/80	90
F. H.	54	N	F	158/110	90	2	146/96	148/98	158/102	84
M. H.	40	N	F	168/128	66	7	118/92	116/90	108/82	68
M. K.	43	C	F	218/116	86	3	168/120	158/106	154/112	69
C. M.	57	N	M	280/140	129	6	180/108	162/104	150/90	86
R. M.	62	C	F	226/116	75	8	176/92	152/76	140/84	84
M. P.	48	N	F	200/120	72	8	122/96	124/96	118/94	80
C. R.	59	C	M	174/94	60	5	140/80	110/75	105/70	72
J. W.	59	N	M	182/122	90	2	148/102	148/104	146/102	84

\* N = Negro; C = Caucasian.

duction in arterial blood pressure occurred between the second and eighth weeks, with no subjective or objective evidence of orthostatic hypotension. The effect of furazolidone on the calculated mean arterial blood pressure was a reduction of  $39 \pm 12$  mm. of mercury.

Various methods were used to determine the basic factor(s) involved in this hypotensive response.

(1) Bacteriological studies of the intestinal flora were made as follows: Each patient had two stool cultures performed before, during, and at the peak of the reduction of blood pressure. No gross changes were observed qualitatively or quantitatively using aerobic and anaerobic or eosin-methylene blue agar plates techniques (table III).

(2) Blood volume determinations were carried out in 3 cases. No change was produced in the blood volumes when arterial blood pressures were reduced.

TABLE III

*Effect of Furazolidone on Intestinal Flora*

Aerobic	Anaerobic	Eosin-methylene blue
Before therapy		
<i>Escherichia coli</i>	Nonhemolytic <i>Streptococcus</i>	<i>Escherichia coli</i>
<i>Staphylococcus aureus</i>	species	<i>Proteus vulgaris</i>
Nonhemolytic <i>Streptococcus</i> species	<i>Pseudomonas</i> species	Nonlactose-fermenters
<i>Pseudomonas</i> species	<i>Clostridium</i> species	
Fungi		
After therapy		
<i>Escherichia coli</i>	Nonhemolytic <i>Streptococcus</i>	<i>Escherichia coli</i>
<i>Staphylococcus aureus</i>	species	<i>Proteus vulgaris</i>
<i>Pseudomonas</i> species	<i>Escherichia coli</i>	Nonlactose-fermenters
<i>Bacillus subtilis</i>		
<i>Proteus vulgaris</i>		
<i>Clostridium</i> species		
Enterococci		

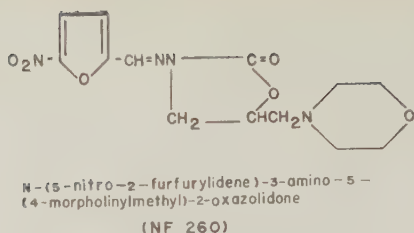


FIGURE 4.

(3) Using the  $\text{Na}^{22}$  method, the sodium space and exchangeable sodium were determined, revealing an increase in sodium excretion with a decrease in both calculated sodium space and total exchangeable sodium. There was no significant change in either serum sodium or potassium.

(4) Study of protein-bound iodine was performed to show the status of the thyroid function. Again no significant change was observed.

(5) Electroencephalographic studies also revealed no change from the control patterns.

(6) Because of the chemical similarity of this nitrofurantoin to certain anticonvulsants (trimethadione), the effect of furazolidone on the electroconvulsion threshold was measured in rabbits. No perceptible changes were observed.

The main side effect of furazolidone was nausea, but after two to four weeks of continuous treatment, this diminished or disappeared and the drug was better tolerated. Nausea could be controlled with prochlorperazine, suggesting that the gastrointestinal disturbance may have a central component in the chemoreceptor trigger zone. However, in many cases, aluminum hydroxide alone was sufficient to modify this gastrointestinal disturbance.

Normocytic anemia of the primaquine type, as has been reported in a few Negroes with nitrofurantoin,<sup>16</sup> was detected also in several of our Negro patients, but was not seen in the Caucasian subjects.

A brownish discoloration of the urine, presumably due to the presence of small amounts of the drug and its metabolites, was noted during therapy. None of the subjects complained of this chromaturia, which was useful as evidence that the drug was being ingested, especially in the management of ambulatory patients.

Another study was undertaken to evaluate the antihypertensive action of a second nitrofurantoin known as NF-260, and which is chemically N-(5-nitro-2-furfurylidene)-3-amino-5-(4-morpholinylmethyl)-2-oxazolidone (fig. 4). Its antibacterial spectrum (table IV) is not so extensive as that of furazolidone. In a preliminary study group of 6 hypertensive patients, a substantial reduction in arterial blood pressure was obtained in 2 patients within a period of three weeks when the drug was administered orally at a dosage of 200 mg. four times a day.

TABLE IV  
The in Vitro Antibacterial Spectrum of NF-260 as Determined by the  
Broth Serial Dilution Method

<i>Streptococcus pyogenes</i>	<i>Salmonella choleraesuis</i>	<i>Clostridium tetani</i>
<i>Streptococcus agalactiae</i>	<i>Salmonella gallinarum</i>	<i>Escherichia coli</i>
<i>Clostridium bifermentans</i>	<i>Salmonella species</i>	<i>Aerobacter aerogenes</i>
<i>Diplococcus pneumoniae</i>	<i>Streptococcus faecalis</i>	<i>Klebsiella pneumoniae</i>
<i>Erysipelothrix rhusiopathiae</i>	<i>Bacillus anthracis</i>	<i>Pseudomonas aeruginosa</i>
<i>Corynebacterium diphtheriae</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas syringae</i>
<i>Mycobacterium smegmatis</i>	<i>Clostridium perfringens</i>	<i>Proteus vulgaris</i>
<i>Vibrio fetus</i>	<i>Clostridium sporogenes</i>	<i>Hemophilus pertussis</i>
<i>Pasteurella avicida</i>	<i>Clostridium histolyticum</i>	<i>Brucella abortus</i>
<i>Salmonella typhosa</i>		

In an attempt to explain the mechanism whereby furazolidone decreases the elevated arterial blood pressure of primary hypertension, several important features must be emphasized. First, the blood pressure of normotensive individuals is unaltered. Second, there is a gradual reduction to a stable plateau, which cannot be lowered further by increased dosage, so that orthostatic hypotension does not occur. Up to eight weeks of continuous medication may be required to produce this effect. Furthermore, once this reduced pressure level is obtained, it is stable and the dosage can be gradually reduced with affecting it.

Quite recently, Kraemer<sup>17</sup> reported a substantial reduction of blood pressure in a group of hypertensive patients treated with a mixture of either penicillin V and trisulfapyrimidine or penicillin G and sulfadimetine.

If furazolidone acts by decreasing sympathetic tonicity, the following hypotheses should be considered:

1. Although the normal intestinal flora is not grossly changed, the possibility remains that certain organisms that liberate pressor amines are eliminated.
2. Biotransformation of the nitrofurans may result in the formation of a compound capable of producing parasympatheticotonia. A survey of the chemical structures of various cholinergic agents reveals that pilocarpine, muscarine, and furtrethonium all contain the furan nucleus.

The classical disulfiram reaction in one of our patients (C. F.) suggests that furazolidone may inhibit in vivo the enzyme system responsible for the conversion of acetaldehyde to acetate. The interruption of various metabolic pathways of carbohydrate metabolism may give us a clue to its hypotensive action.

The result of prolonged metabolic studies indicates a decrease in total exchangeable sodium space and an increase in renal excretion of sodium without alteration of serum sodium and potassium. The observations require further study, since no change was observed in blood volume.

To consider simply this vasodepressor action a function of the nitro group, as found in the organic nitrates, is not warranted at this time, since continuous intravenous infusion of nitrofurans into normal dogs failed to elicit a vasomotor response.

#### SUMMARY

It was demonstrated that when nitrofurans (NF-180 and NF-260) were administered by mouth there was produced a slow, gradual reduction of arterial blood pressure in adults with uncomplicated primary hypertension. The time required for this response, when furazolidone (NF-180) was used, varied from two to eight weeks. This drug is poorly absorbed from the intestinal tract and systemic toxicity is mild except for nausea. The mode of action is still under investigation and several possible mechanisms have been discussed.

#### BIBLIOGRAPHY

1. LEPLEY, K. C.; CATRON, D. V., AND CULBERTSON, C. C.: Dried whole aureomycin mash and meat and bone scraps for growing-fattening swine, *J. Animal Sc.* 9:608, 1950.
2. CARPENTER, L. E.: Effect of aureomycin on the growth of weaned pigs, *Arch. Biochem.* 27:469, 1950.
3. SAXENA, H. C.; BERG, L. R., AND MCGINNIS, J.: Factors affecting the growth response of chick and turkey poults to antibiotics, *Poultry Sc.* 31:1070, 1952.

4. FANGAUF, R., AND VON BARLOEWEN, G.: Wachstumseffekt bei Nitrofurazon-Gaben, *Arch. Gefluegelk.* 18:29, 1954.
5. VOELKER, H. H., AND CASON, J. L.: Antibiotics studies with dairy calves, *J. Animal Sc.* 10:1065, 1951.
6. JUKES, T. H.: Antibiotics in Nutrition. New York, Medical Encyclopedia, Inc., 1955.
7. PRIDGEN, J. E.: Respiratory arrest thought to be due to intraperitoneal neomycin, *Surgery* 40:571, 1956.
8. POTH, E. J.: Critical analysis of intestinal antisepsis, *J.A.M.A.* 163:1317, 1957.
9. GABUZDA, G. J.; GOCKE, T. M.; JACKSON, G. G.; GRIGSBY, M. E.; LOVE, B. D., JR., AND FINLAND, M.: Some effects of antibiotics on nutrition in man, *A.M.A. Arch. Int. Med.* 101:476, 1958.
10. CALESNICK, B., AND DiPALMA, J. R.: The hypotensive effects of furoxone, *J. Pharmacol. & Exper. Therap.* 122:1, 1958.
11. YURCHENCO, J. A.; YURCHENCO, M. C., AND PIEPOLI, C. R.: Antimicrobial properties of furoxone (N-5-nitro-2-furfurylidene-3-amino-2-oxazolidone), *Antib. & Chemo.* 3:1035, 1953.
12. ROGERS, G. S.; BELLOFF, G. B.; PAUL, M. F.; YURCHENCO, J. A., AND GEVER, G.: Furozolidone, a new antimicrobial nitrofurantoin: a review of laboratory and clinical data, *Antib. & Chemo.* 6:231, 1956.
13. BENDER, R. C., AND PAUL, H. E.: Metabolism of the nitrofurans: incubation of furacin with mammalian tissues, *J. Biol. Chem.* 191:217-222, 1951.
14. PAUL, M. F.; PAUL, H. E.; KOPO, F.; BRYSON, M. J., AND HARRINGTON, C.: Inhibition by furacin of citrate formation in testis preparation, *J. Biol. Chem.* 206:491-497, 1953.
15. TAYLOR, J. D.; PAUL, H. E., AND PAUL, M. F.: Metabolism of the nitrofurans: Studies with xanthine oxidase in vitro, *J. Biol. Chem.* 191:223-231, 1951.
16. KIMBRO, E. L.; SACHS, M. V., AND TORBERT, J. V.: Mechanism of the hemolytic anemia induced by nitrofurantoin (Furadantin), *Bull. Johns Hopkins Hosp.* 101:245, 1957.
17. KRAEMER, E.: Ueberraschende blutdrucksenkende wirkung der penicillin-sulfonamid-praeparate "Sulfaoratren" and "Aristocillin" bei fixierten hypertoniem, *München. med. Wchnschr.* 100:1004, 1958.

# Furmethonol—a New Antibacterial Nitrofuran

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The increasing medical problems associated with *Staphylococcus aureus*, particularly drug-resistant strains of this species, have led to the exploration of various control measures. Perhaps the most intense activity has been directed toward the development of new drugs, always with at least one major objective being to provide effective chemotherapeutic agents of such a nature as to minimize the emergence of drug-resistant bacterial strains. This report describes the laboratory bacteriological evaluation of furmethonol,<sup>1</sup> N-(5-nitro-2-furfurylidene)-3-amino-5-(N'-morpholinyl-methyl)-2-oxazolidone, a new nitrofuran selected and developed in the course of screening more than 100 nitrofuran compounds against antibiotic-resistant staphylococci.

## MATERIALS AND METHODS

*Animals.* CFW white male mice weighing 18 to 20 Gm. were used. The animals were caged in groups of five and given food and water ad libitum.

*In Vitro Studies.* Sensitivity determinations were carried out by the broth dilution method and the disc method. Brain-heart infusion (Difco) was employed for the broth dilution sensitivity tests. The broth was supplemented with 0.2 per cent agar for tests with *Vibrio* and *Clostridium*. The inocula for the broth dilution tests were obtained from 16 hour brain-heart infusion agar slants (Difco). Cells from the slants were washed, suspended, and diluted in Ringer's solution to provide an inoculum of  $1.5 \times 10^4$  cells contained in 0.1 ml. The inocula for tests employing *Clostridium* or *Vibrio* were prepared by making a 1:1000 dilution of 24 hour cultures grown in brain-heart infusion containing 0.2 per cent agar. Furmethonol solutions were prepared by dissolving the compound in broth or distilled water and Seitz filtering the solution. Concentrations were determined spectrophotometrically.

Disc sensitivity tests were performed using blood agar plates, which had been streaked with cotton swabs from suspensions containing approximately  $2 \times 10^7$  cells. The antibiotic sensitivity discs employed were Sensi-discs (BBL).

Furmethonol- and erythromycin-resistant strains of *Staph. aureus* were obtained by the daily transfer of the organisms in brain-heart infusion containing increasing drug concentrations.

*MOUSE KIDNEY CULTURE.* Mice were sacrificed by the use of ether and the kidneys were removed aseptically and rinsed in sterile saline. Each kidney was then placed in a sterile 5 ml. syringe fitted with a 40 mesh stainless steel grill and a 3½ inch 15 gauge needle. Homogenizing was accomplished by forcing the tissue through the grill and needle into 5 ml. of brain-heart infusion. The broth tubes were incubated for 24 hours at 37 C. after which time aliquots were streaked onto *Staphylococcus* medium no. 110 (Difco), or brain-heart infusion agar containing 7 per cent sodium chloride. After 48 hours' incubation at 37 C., typical colonies were examined for coagulase production and drug sensitivity.

*VELOCITY OF ANTIBACTERIAL ACTION.* 50 ml. aliquots of brain-heart infusion containing selected furmethonol concentrations were inoculated with cells washed from 16 hour slants. After incubation at 37 C. plate counts were performed at appro-

TABLE I

*The Sensitivity of Various Bacterial Genera to Furmethonol*

Bacterial genera	No. of strains	Range of 24 hour minimal inhibiting concentration, $\mu\text{g.}/\text{ml.}^*$
<i>Staphylococcus</i>	140	3-9
<i>Streptococcus</i> A	6	10-40
B	3	20-200
D	3	4-20
<i>Diplococcus</i>	1	3
<i>Bacillus</i>	3	1-2
<i>Erysipelothrix</i>	4	10-50
<i>Corynebacterium</i>	1	2
<i>Clostridium</i>	6	1-4
<i>Mycobacterium</i>	1	>120
<i>Escherichia</i>	6	2-20
<i>Klebsiella</i>	4	30-240
<i>Salmonella</i>	15	7-30
<i>Pseudomonas</i>	7	190->390
<i>Proteus</i>	6	390->390
<i>Pasteurella</i>	4	5-20
<i>Brucella</i>	1	190
<i>Vibrio</i>	4	.05-1

\* Broth dilution test.

priate intervals up to 24 hours. Sterilization of the cultures was demonstrated by filtration through membrane filters.<sup>2</sup>

Coagulase determinations were performed with the use of desiccated plasma (Difco).

*In Vivo Studies.* Chronic infections were established by the intravenous injection of approximately  $2 \times 10^8$  cells (Smith strain of *Staph. aureus*). The strain was maintained on brain-heart infusion agar and transferred in broth prior to the preparation of the inoculum from an 18 hour culture grown in brain-heart infusion.

Acute infections were established by the intraperitoneal injection of  $6 \times 10^6$  cells suspended in 5 per cent hog gastric mucin (Wilson). The strain employed was *Staph. aureus* (Mi-12). This is a hemolytic, coagulase-positive, penicillin-resistant strain isolated from a fatal septicemia.

*Drug Administration.* All drugs were suspended or dissolved in 0.75 per cent carboxymethylcellulose (Hercules) and administered to mice in 0.5 ml. volumes by gavage.

TABLE II

*The in Vitro Sensitivity of 100 Strains of Staphylococcus aureus to Furmethonol and 6 Antibiotics\**

Agent	Disc conc., $\mu\text{g.}$	No. of sensitive strains
Furmethonol	30	100
Carbomycin	15	100
Chloramphenicol	30	98
Erythromycin	15	89
Chlortetracycline	30	64
Penicillin	10 units	51
Streptomycin	50	40

\* Streak plate method.

TABLE III

*The Effect of Inoculum Size on the Activity of  
Furmethonol Against Staphylococcus aureus (Mi-6)\**

Cell inoculum	Minimal inhibiting concentration, $\mu\text{g.}/\text{ml.}$			
	24 hr.	48 hr.	72 hr.	96 hr.
$5.6 \times 10^7$	24	24	24	24
$5.6 \times 10^6$	6	6	6	6
$5.6 \times 10^5$	3	6	6	6
$5.6 \times 10^4$	3	3	3	3
$5.6 \times 10^3$	3	3	3	3

\* Serial twofold broth dilution method.

## RESULTS AND DISCUSSION

**Antibacterial Spectrum.** Furmethonol sensitivity was determined for 215 bacterial strains representing 16 genera. The results presented in table I indicate that the most sensitive gram-positive organisms are *Staphylococcus*, *Diplococcus*, *Bacillus*, *Corynebacterium*, and *Clostridium*, while *Escherichia*, *Vibrio*, *Pasteurella*, and *Salmonella* are considered the sensitive gram-negative genera.

**Sensitivity of *Staphylococcus aureus*.** Table II presents the results obtained from the sensitivity testing (disc method) of 100 recent isolates of *Staph. aureus*. It may be seen that 100 per cent of the strains were sensitive to furmethonol irrespective of their sensitivity to the six antibiotics. Sensitivity to the antibiotics varied from 100 per cent with carbomycin to 40 per cent with streptomycin.

**Effect of Inoculum Size on the Activity of Furmethonol.** It has been reported by several workers<sup>3-5</sup> that the sensitivity of various bacterial species to nitrofurantoin\* varies with the size of the inoculum employed in broth dilution tests. This effect has been noted in our laboratory for a variety of nitrofurans and is demonstrated for furmethonol by the results shown in table III. Replicate dilution series of furmethonol were prepared and inoculated with decimal dilutions of an antibiotic sensitive *Staph. aureus*. The tubes were incubated at 37 C. and examined for visual growth at 24 hour intervals up to 96 hours. The results indicate that an inoculum effect does exist, as evidenced by the eightfold increase in sensitivity resulting when the inoculum is increased from  $10^3$  to  $10^7$ .

Although it has been postulated by Waisbren<sup>5</sup> that the inoculum effects observed with nitrofurantoin are related to "inhibitors" produced by the bacteria, no similar studies have been performed with furmethonol.

**Rate of Antibacterial Action of Furmethonol.** It has been shown that at selected concentrations nitrofurantoin, nitrofurazone,† and furazolidone‡ exert a bactericidal action.<sup>3,6,7</sup> Figure 1 illustrates that furmethonol was bactericidal at concentrations of 6 and 12  $\mu\text{g.}/\text{ml.}$  while the lowest concentration (2  $\mu\text{g.}/\text{ml.}$ ) was bacteriostatic for 12 hours when a rapid increase in the viable count occurred. Although sterility was accomplished in 24 hours, viable cells were still present after eight hours exposure to the compound. These results are in agreement with those reported for furazolidone by Yurchenco et al.<sup>7</sup>

**Resistance Studies.** An antibiotic sensitive strain of *Staph. aureus* (Mi-67) was employed in order to compare the rates of development of resistance to furmethonol

\* The trade name of Eaton Laboratories for nitrofurantoin is Furadantin.

† The trade name of Eaton Laboratories for nitrofurazone is Furacin.

‡ The trade name of Eaton Laboratories for furazolidone is Furoxone.

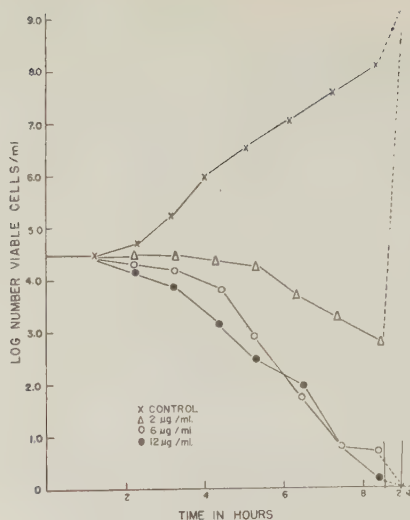


FIG. 1. The effect of three concentrations of furmethonol on cultures of *Staphylococcus aureus* (Mi-6) is shown.

and erythromycin. After 10 transfers in broth containing increasing drug concentrations the resistance of the strain to erythromycin had increased 160-fold, while the increase in resistance to furmethonol was 12-fold. These results are illustrated in figure 2.

A penicillin-resistant strain of *Staph. aureus* (Mi-12) was made resistant to furmethonol in order to explore the possibility of a simultaneous increase in resistance to antibiotics. Broth dilution sensitivity tests were then carried out on both the furmethonol-sensitive parent strain and the resistant strain (table IV). The results show that the antibiotic sensitivity of the two strains is identical, so that for this particular strain no simultaneous increase in antibiotic resistance could be shown. These results are similar to those reported by Kefauver et al<sup>8</sup> for furazolidone.

*Protective Studies in Mice.* Table V presents the results obtained after treatment of various groups of infected mice with furmethonol, erythromycin, or oxytetracycline. For these experiments, mice were injected intraperitoneally with a lethal dose of *Staph. aureus* (Mi-12) in 5 per cent mucin. When treated with a single dose, 0.5 hour postinfection, there was no significant difference between the activity of furmethonol and erythromycin, while oxytetracycline was more effective than either. Similarly, there is little difference between the ED<sub>50</sub> figures obtained for furmethonol

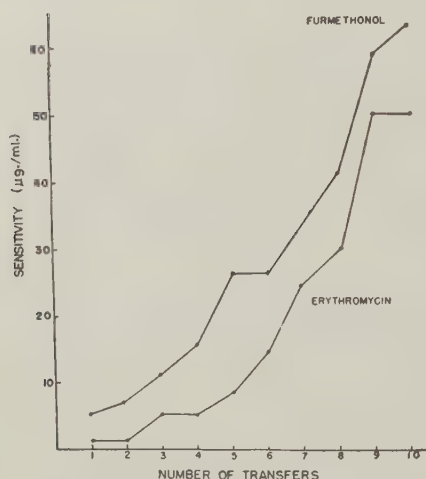


FIG. 2. The rate of development of resistance to furmethonol and erythromycin by *Staphylococcus aureus* (Mi-67) is given.

TABLE IV

*The Sensitivity to Antibiotics of Staphylococcus aureus (Mi-12)  
Parent Strain and the Furmethonol-Resistant Mutant*

Agent	24 hour minimal inhibiting concentration, $\mu\text{g./ml.}$	
	Parent strain	Furmethonol-resistant strain
Furmethonol	8	50
Chlortetracycline	0.2	0.2
Oxytetracycline	1.2	1.2
Chloramphenicol	20	10
Streptomycin	10	10
Erythromycin	2.5	2.5

and erythromycin when the drugs were administered in three equal doses 0.5, 4.5 and 8 hours postinfection, or in a single dose at 4 hours postinfection.

*Furmethonol Evaluation by Mouse Kidney Culture.* Mice infected by the intravenous route with the Smith strain of *Staph. aureus* were treated with furmethonol and novobiocin at 50 mg./Kg./day for seven days. The results presented in table VI show that furmethonol treatment was more effective than novobiocin treatment whether the treatment was commenced 36 hours or seven days after inoculation. In both cases the number of mice harboring *Staphylococcus* was significantly lower in the furmethonol treated groups than in the novobiocin treated groups. Similarly, mice infected intraperitoneally with *Staph. aureus* (Mi-12) were treated 30 minutes after inoculation with furmethonol or erythromycin. Irrespective of whether the mice were treated at a level of 25 or 50 mg./Kg./day or whether they were sacrificed at 7, 14, or 53 days, the number of isolates from furmethonol treated groups was always less than the number from the erythromycin treated groups.

It is generally agreed that the experimental, chronic infection<sup>10,11</sup> produced in mice by the intravenous inoculation of the Smith strain of *Staphylococcus aureus* allows a more definitive drug evaluation than is possible with acute infections produced by intraperitoneal inoculation. It would appear that the ability of a drug to eradicate completely the organism from the infected kidney is a more stringent and a more practical measure of drug efficacy than is the comparison of viable counts. Information gained by the former method, in addition to providing an indication of the bacteriostatic versus bactericidal nature of the compound, is more easily translated to practical conditions of treatment. For example, bacterial cell counts ob-

TABLE V

*The Efficacy of Furmethonol and 2 Antibiotics in Acute Staphylococcal Infection of Mice*

Agent	No. treatments	Time, hr.	ED <sub>50</sub> *, mg./Kg.	95 per cent confidence limits	No. of mice
Furmethonol	1	0.5	22.2	19.7 -25.1	410
Erythromycin	1	0.5	23.0	20.5 -25.8	195
Oxytetracycline	1	0.5	6.86	4.88- 9.64	125
Furmethonol	3	0.5-4.5-8	32.7	26.4 -40.4	120
Erythromycin	3	0.5-4.5-8	47.8	38.9 -58.7	65
Furmethonol	1	4	66.8	53.9 - 82.9	120
Erythromycin	1	4	81.5	54 -123	80

\* Method by Bliss.<sup>9</sup>

TABLE VI

*Mouse Kidney Retention of Staphylococcus aureus after Furmethonol  
or Antibiotic Treatment*

Agent	Initial treatment	Total drug, mg./Kg.	Culture days postinfection	No. positive mice/total	% positive
<i>Intravenous Infection—Smith strain*</i>					
Furmethonol hydrochloride	36 hr.	750	9	5/28	18
Novobiocin	36 hr.	750	9	14/30	47
Control	—	—	9	26/28	93
Furmethonol hydrochloride	7 days	750	14	7/27	26
Novobiocin	7 days	750	14	13/26	50
Control	—	—	14	23/30	77
<i>Intraperitoneal Infection—Strain Mi-12†</i>					
Furmethonol	0.5 hr.	50	7	3/20	15
	0.5 hr.	50	14	0/20	0
	0.5 hr.	50	53	0/18	0
Erythromycin	0.5 hr.	50	7	9/19	47
	0.5 hr.	50	14	5/20	25
	0.5 hr.	50	53	2/18	11
Furmethonol	0.5 hr.	25	7	12/20	60
	0.5 hr.	25	14	5/20	25
	0.5 hr.	25	53	1/20	5
Erythromycin	0.5 hr.	25	7	17/20	85
	0.5 hr.	25	14	10/20	50
	0.5 hr.	25	53	5/20	25

\* Drugs administered by gavage every 12 hours for seven days.

† Single drug doses by gavage 0.5 hour after infection.

tained from the kidneys of comparable groups of treated animals may indicate definitely the superiority of one drug over another. On the other hand, this information cannot readily be translated to practical circumstances unless one is prepared to assume that reductions in bacterial counts are synonymous with cures. The results shown in table VI indicate that both furmethonol and novobiocin were able to eradicate the infection in a significant number of mice. Similarly, it has been demonstrated<sup>8</sup> that the occurrence of gross lesions of the kidneys does not give an accurate indication of infection. It has been possible to recover *Staph. aureus* from kidneys showing no gross pathology and, conversely, bacteria could not be demonstrated in some kidneys showing gross lesions. Similar observations have been made by Wilkins et al.<sup>12</sup>

It was shown that the organisms isolated from the kidneys of treated animals had retained their sensitivity to furmethonol and the antibiotics so that treatment failure must be ascribed to factors other than the development of resistance.

#### SUMMARY

The in vitro and in vivo bacteriological evaluation of furmethonol was described. The drug was shown to meet the bacteriological requirements for further development as a potentially useful chemotherapeutic agent in the treatment of *Staphylococcus aureus* infections.

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## BIBLIOGRAPHY

1. U. S. pat. No. 2802002.
2. HOLDOWSKY, S.: A new sterility test for antibiotics. An application of the membrane filter technique, *Antib. & Chemo.* 7:49, 1957.
3. WAISBREN, B. A., AND CROWLEY, W.: Nitrofurantoin: Clinical and laboratory evaluation, *A.M.A. Arch. Int. Med.* 95:653, 1955.
4. RICHARDS, W. A.; RISS, E.; KASS, E., AND FINLAND, M.: Nitrofurantoin: Clinical and laboratory studies in urinary tract infections, *A.M.A. Arch. Int. Med.* 96:437, 1955.
5. WAISBREN, B. A.: A *Proteus* produced inhibitor to nitrofurantoin, *Antib. & Chemo.* 7:586, 1957.
6. CRAMER, D. L., AND DODD, M.C.: The mode of action of nitrofuran compounds. I. Action versus *Staphylococcus aureus*, *J. Bact.* 51:293, 1946.
7. YURCHENCO, J. A.; YURCHENCO, M. C., AND PIEPOLI, C. R.: Antimicrobial properties of Furoxone (N-5-nitro-2-furfurylidene-3-amino-2-oxazolidone), *Antib. & Chemo.* 3:1035, 1953.
8. KEFAUVER, D. F.; McRAE, A., AND O'CONNOR, J. R.: The sensitivity of antibiotic resistant *Micrococcus pyogenes* to furazolidone (N-5-nitro-2-furfurylidene-3-amino-2-oxazolidone), *Bact. Proc.* 1956, p. 64.
9. BLISS, C. I.: A comparison of dosage-mortality data, *Ann. Applied Biol.* 22:307, 1935.
10. SMITH, J. M., AND DUBOS, R. J.: The behavior of virulent and avirulent staphylococci in the tissues of normal mice, *J. Exper. Med.* 103:87, 1956.
11. GORRILL, R. H.: Experimental staphylococcal infections in mice, *Brit. J. Exper. Path.* 32:151, 1951.
12. WILKINS, J. R.; GRAY, J. E.; NIKITAS, C. T., AND PRESTRUD, M. C.: The effect of a new antibiotic, novobiocin, on the course of an experimental *Staphylococcus* infection in mice. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 1063.

# The Action of a New Steroid Acid Amide of Diaminodiphenyl Sulfone on the PR8 Strain of Influenza Virus A in the Chick Embryo

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In a previous communication,<sup>1</sup> evidence was presented on the marked antitubercular action and perfect acceptability by human beings and experimental animals of certain steroid acid amides of diaminodiphenyl sulfone. These compounds, as well as a few new steroid derivatives that I synthesized, were tested for antiviral activity on the PR8 strain of influenza virus A in the chick embryo. 4-(3-Hemisuccinyl desoxycholylamino)-4'-hemisuccinylamino diphenyl sulfone (B139) showed noteworthy activity. A brief survey of the experimental findings is presented.

The method of the preparation of B139 will be published elsewhere. The infrared spectrum of a crystalline sample (fig. 2) is consistent with the structural formula, shown in figure 1. B139 is soluble in alcohol, acetone, and propylene glycol. It is sparingly soluble in water. However, alkali metal and amine salts are water soluble.

## MATERIALS AND METHODS

Fertile white Leghorn eggs, which had been preincubated for 11 days, were used.

*Virus Inocula.* Virus-infected allantoic fluid, frozen and refrigerated in the dry ice chest, was used. The thawed contents of two to three vials were pooled for each experiment and showed a titer of 1500 to 2000 hemagglutinating units/ml. Tenfold serial dilutions were made in phosphate buffer. The inocula consisted of 0.1 ml. of either  $10^{-3}$ ,  $10^{-4}$ , or  $10^{-5}$  dilutions containing estimated<sup>2,3</sup>  $10^5$ ,  $10^4$ , or  $10^3$  infective doses, respectively. Injections were made into the allantoic cavity.

*Drug.* B139 was freshly dissolved in a sterile tube by first carefully adding N/20 sodium hydroxide in small portions. Water was added to make a final drug concentration of 2 per cent at a pH 7.7 to 7.9. Treatment was administered by injecting 0.05, 0.1, or 0.2 ml. drug solution containing 1, 2, or 4 mg. B139 into the allantoic cavity either  $\frac{1}{2}$  to two hours before or, in the majority of experiments, a few minutes after virus inoculation. Virus-inoculated but nontreated eggs were included in each experiment in at least equal number to the eggs in the single therapeutic test.

*Virus Titration.* After incubation of the eggs for 40 to 44 hours at 37 C., virus concentration in the allantoic fluid of each egg was determined separately by the method of chicken erythrocyte agglutination<sup>4</sup> (pattern test<sup>5</sup>). Twofold serial dilutions of the allantoic fluids were made in normal saline. To the tubes containing 0.5 ml. of plain or diluted fluid, 0.5 ml. of washed chicken erythrocytes in saline ( $\frac{1}{2}$  per cent) was added. As a rule, the tubes could be read after standing for one hour at room temperature.

## EXPERIMENTAL RESULTS AND DISCUSSION

The findings on 143 treated eggs and 76 inoculated nontreated eggs are tabulated in table I and further illustrated by the graphs in figures 3, 4, and 5.

A brief description of table I follows. Data on groups and subgroups of eggs are shown in the rows. There are two main divisions: the drug-treated eggs at the top,

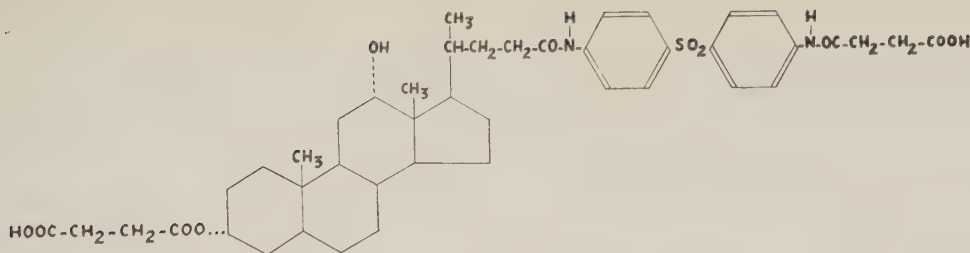


FIG. 1. The chemical structure of 4-(3-hemisuccinyl desoxycholylamino)-4'-hemisuccinylamino diphenyl sulfone (B 139) is shown.

the nontreated inoculated controls at the bottom. Both main divisions are comprised of three groups according to the dilutions of the stock virus used for the inocula, namely, the groups of  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$ . For the control eggs, this is the final grouping. In the division of treated eggs, each of the three groups is again subdivided according to the drug dosage administered, namely, 4, 2, or 1 mg. Finally, some of these subgroups are again divided according to the time of drug administration, namely, into the ultimate subgroups referring to treatment either before (A) or after (P) virus inoculation.

The columns include data on procedure and findings of virus titration as done on the allantoic fluids obtained from the eggs. The main columns correspond to the progressive dilutions in the eight tubes of the typical test. In each column, there are three subdivisions for the grading of the agglutination.

Table I presents the numerical distribution of the results of hemagglutination of the fluids in each group of eggs and in each of the eight dilutions. These data are also expressed as per cent of the total number of eggs in the groups. However, for this purpose, the negatives (O) and partials ( $\pm$ ) were added, calculated, and listed in one column, and the positives are in another column.

The effect of treatment on massive infection (inoculum  $10^5$  infective doses) was investigated in more detail. Observations on 125 eggs, 86 treated and 39 infection controls, were recorded. All untreated controls showed positive agglutination up to the dilution 1:128 (256); at 1:256 (512), 29 of 39 were positive; 9 had a titer greater than 1:1000 (2000). Against this background even a comparatively small number of treated eggs with negative undiluted fluids can safely be considered significant. With 4 mg. administered before virus inoculation, 5 of 20 (25 per cent) plain fluids were negative. Seven (35 per cent) had a titer of less than 1:16 (32). When treated

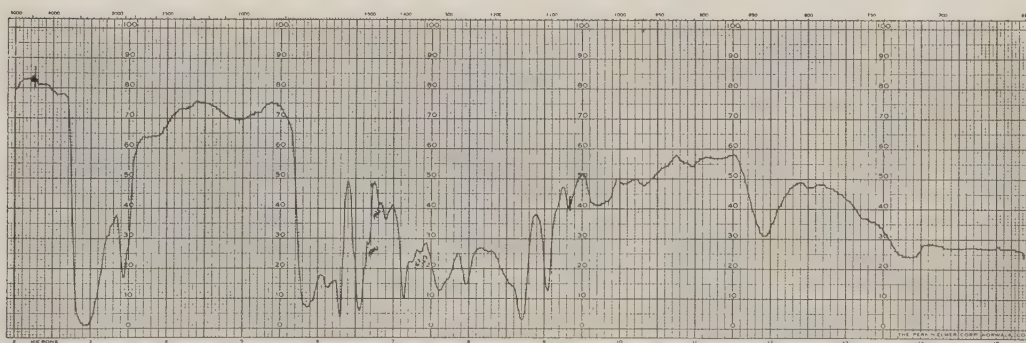


FIG. 2. The infrared spectrum of 4-(3-hemisuccinyl desoxycholylamino)-4'-hemisuccinylamino diphenyl sulfone (B 139) is illustrated.

TABLE I  
Virus Titration of Allantoic Fluids by

Dilution of stock virus	Dilution of allantoic fluids									
	0 (2)		16 (32)			32 (64)			64 (128)	
	0	± +	0	± +		0	± +		0	± +
10 <sup>-3</sup> (Dose: 4 mg./Egg)										
A° No. fluids	5	15	7	13		9	10	11	4	5
Per cent	25	75	35	65		50	50	75	25	
P° No. fluids	3	27	6	23		9	3	20	13	3
Per cent	15.62	84.38	28.12	71.88		37.5	62.5	50	50	
10 <sup>-3</sup> (Dose: 2 mg./Egg)										
A No. fluids	1	8	1	8		1	8	1	3	5
Per cent	11.11	88.89	11.11	88.89		11.11	88.89	44.44	55.56	
P No. fluids		16	1	15		1	15	1	15	
Per cent		100	6.25	93.75		6.25	93.75	6.25	93.75	
10 <sup>-3</sup> (Dose: 1 mg./Egg)										
A No. fluids		5		5			5		5	
P No. fluids		4		4			4	1	3	
Per cent A&P		100		100			100	11.11	88.89	
10 <sup>-4</sup> (Dose: 4 mg./Egg)										
P No. fluids	4	4 12	14	2 4	16		4	16		4
Per cent	40	60	80	20	80		20	80		20
10 <sup>-4</sup> (Dose: 2 mg./Egg)										
P No. fluids	3	1 4	3	2 3	4		2 2	5	1 2	
Per cent	50	50	62.5	37.5	75		25	75	25	
10 <sup>-5</sup> (Dose: 4 mg./Egg)										
P No. fluids	3	5 12	16	2 2	18		2 18	1	1	
Per cent	40	60	90	10	90		10	95	5	
10 <sup>-5</sup> (Dose: 2 mg./Egg)										
P No. fluids	3	6	4	1 4	4		4 1	8	1	
Per cent	33.33	66.67	55.55	44.44	88.89		11.11	88.89	11.11	
Total No. fluids	22	12 109	52	10 81	62		10 71	74	12 57	
Per cent	23.77	76.23	43.35	56.65	50.35		49.65	60.13	39.87	
10 <sup>-8</sup> (Controls Inoculated Not Treated)										
No. fluids		39		39			39		39	
Per cent		100		100			100		100	
10 <sup>-4</sup> (Controls Inoculated Not Treated)										
No. fluids		19		1 18	1		18	1	18	
Per cent		100	5.26	94.74	5.26		94.74	5.26	94.74	
10 <sup>-5</sup> (Controls Inoculated Not Treated)										
No. fluids	1	3 14	4	14	4		14	4	14	
Per cent	22.22	77.78	22.22	77.78	22.22		77.78	22.22	77.78	
Total No. fluids	1	3 72	4	1 71	5		71	5	71	
Per cent	5.26	94.34	6.57	93.43	6.57		93.43	6.57	93.43	

° A, Drug administered ½ to 2 hours before virus inoculation; P, drug administered 2 to 3 minutes after virus

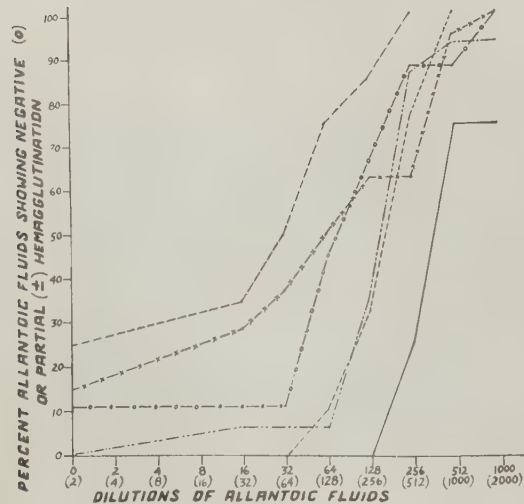


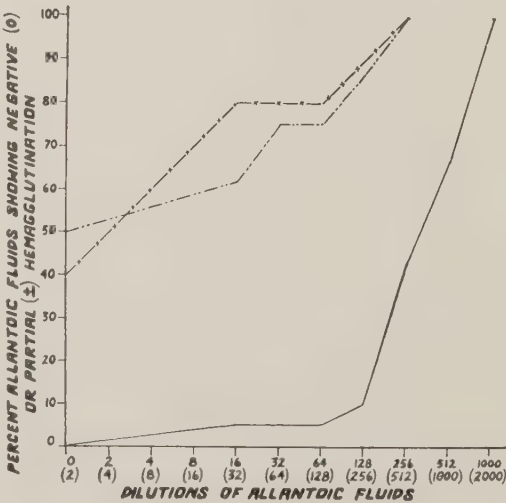
FIG. 3. The inoculum consisted of 0.1 ml. of 10<sup>-3</sup> dilution of stock virus containing estimated 10<sup>5</sup> infective doses. ———, treated with 4 mg. before inoculation; -x-x-, treated with 4 mg. after inoculation; -o-o-, treated with 2 mg. before inoculation; —. —., treated with 2 mg. after inoculation; - - - -, treated with 1 mg. before and after inoculation; —, control eggs inoculated, not treated.

TABLE I  
*Hemagglutination of Treated and Control Eggs*

tested: initial (final)														No. of eggs	No. of experi- ments
128 (256)			256 (512)			512 (1000)			1000 (2000)						
0	±	+	0	±	+	0	±	+	0	±	+				
15	2	3	18	2		20			20			20	4		
85		15	100			100			100						
17	4	11	17	4	11	28	2	2	30	2		32	8		
62.5		37.5	62.5		37.5	93.75		6.25	100						
2	4	3	6	2	1	7	1	1	9			9	2		
66.67		33.33	88.89		11.11	88.89		11.11	100						
3	3	10	12	2	2	15		1	15		1	16	4		
37.5		62.5	87.5		12.5	93.75		6.25	93.75		6.25				
1		4	5			5			5			5	1		
2		2	2		2	4			4			4	1		
33.33		66.67	77.78		22.22	100			100						
16	2	2	18	2		18	2		20			20	4		
90		10	100			100			100						
6	1	1	8			8			8			8	2		
87.5		12.5	100			100			100						
19	1		20			20			20			20	4		
100			100			100			100						
8		1	8		1	9			9			9	2		
88.89		11.11	88.89		11.11	100			100						
89	17	37	114	12	17	134	5	4	140	2	1	143			
74.13		25.87	88.12		11.88	97.21		2.79	99.30		0.7				
		39	5	5	29	15	15	9	30		9	39	8		
		100	25.64		74.36	76.92		23.08	76.92		23.08				
1	1	17	4	4	11	7	6	6	13	6		19	4		
10.53		89.47	42.11		57.89	67.9		32.1	100						
5		13	6	4	8	12	3	3	17		1	18	4		
27.77		72.23	55.55		44.45	83.34		16.66	94.45		5.55				
6	1	69	15	13	48	34	24	18	60	6	10	76			
9.2		90.8	36.84		63.16	76.32		23.68	86.65		13.15				

inoculation.

FIG. 4. The inoculum consisted of 0.1 ml. of  $10^{-4}$  dilution of stock virus containing estimated  $10^4$  infective doses. -x-x-, treated with 4 mg. after inoculation; —. —. —, treated with 2 mg. after inoculation; —, control eggs inoculated, not treated.



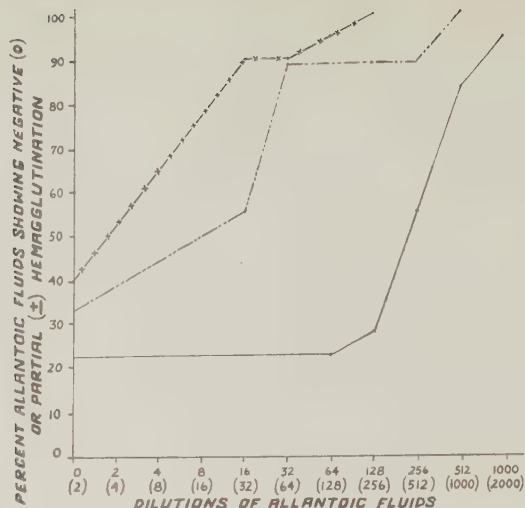


FIG. 5. The inoculum consisted of 0.1 ml. of  $10^{-5}$  dilution of stock virus containing estimated  $10^3$  infective doses. -x-x-, treated with 4 mg. after inoculation; —. —. —, treated with 2 mg. after inoculation; —, control eggs inoculated, not treated.

with 4 mg. after inoculation, 5 of 32 undiluted fluids (15.62 per cent) were practically negative and 9 (28.12 per cent) had a titer of less than 1:16 (32). With treatment of 2 mg. before inoculation, 1 of 9 plain fluids (11.11 per cent) was negative. When 2 mg. was given after inoculation, only 1 of 16 had a titer of less than 1:16 (32). In the eggs treated with 1 mg., some drug activity was detectable only in the higher dilutions. It is interesting to note that in the dilution of 1:128 (256), when all 39 controls were positive, in the treated groups the negatives ranged as follows: 1 mg., 33.33 per cent; 2 mg. treated after inoculation, 37.5 per cent; treated before, 66.67 per cent; 4 mg. treated after, 62.5 per cent; treated before, 85 per cent. In the next higher dilution, when there were 25.64 per cent negatives among the controls, the negatives among the treated ranged from 62.5 to 100 per cent. As stated previously, in the 4 mg. treated series (4 mg. A), 25 per cent of the undiluted fluids were negative. Among controls, at a dilution of 1:256 (512) an almost equal percentage (25.62) were negative. The ratio of the two dilutions would assign an "efficiency index" of 99.6 per cent to the 4 mg. A group. Similarly calculated values might be useful for purposes of comparison.

Treatment done before virus inoculation appears to have a slight edge over treatment done after inoculation. However, further experience is necessary before this difference can be accepted as significant. The presented evidence proves a suppressive effect of B139 on massive virus infection in the chick embryo.

In the experiments done on eggs inoculated with  $10^4$  infective doses (stock virus dilution  $10^{-4}$ ), the results were more conspicuous. A total of 47 eggs, 28 treated and 19 infection controls, were observed in these series. In all controls, the undiluted fluids were positive for agglutination. Only 1 of 19 controls had a titer of less than 1:16. At the dilution 1:128 (256), 2 of 19 were practically negative. At 1:256 (512), 11 of 19 were still positive, and at 1:512 (1000), one third were positive. The controls provided a good base line for the experiments. Treatment was administered after inoculation. In the 4 mg. group, 8 of 20 undiluted fluids (40 per cent) were negative. Sixteen of 20 (80 per cent) showed a titer of less than 1:16 (32). In the group treated with 2 mg., the antiviral action was essentially similar although slightly decreased. These results show an almost uniform suppression of virus multiplication in the chick embryo.

In the series of experiments done on eggs inoculated with  $10^3$  infective doses

(stock virus dilution  $10^{-5}$ ), the results were essentially similar to those obtained in the preceding groups. A total of 47 eggs, 29 treated and 18 infection controls, were used. The results are shown in table I and illustrated in the graph of figure 5.

In a small experimental series done with the presently used system,<sup>6,7</sup> the effect of B139 compared favorably with that of an improved halogenated benzimidazole, namely, 4, 5, 6, (5, 6, 7)-trichloro-1- $\beta$ -D-ribofuranosyl benzimidazole.

In a few tests done in the course of these experiments, the parent sulfone, 4-4' diaminodiphenyl sulfone, and 4-amino 4'-nitro diphenylsulfone administered in dosages of 1 to 4 mg./egg were both toxic and ineffective.

It was recently reported<sup>8</sup> that some sulfones had inhibitory effects on poliomyelitis in monkey testicular explant culture. No appreciable activity was found in HeLa cultures.

Systematic toxicity studies for B139 will be done as soon as larger quantities of the compound are available. In tentative tests for tolerance, up to 15 mg./egg (highest amount tried) were injected into the allantoic cavity without harmful effect.

The results show significant inhibition of virus multiplication by B139, although differing in degree, in all three grades of infection and with any of the three drug dosages administered in the two treatment schedules. The results were unexpected, since other sulfones and sulfonamides were repeatedly proved ineffective in clinical and experimental influenza infection.<sup>9</sup> It has also been demonstrated that a steroid such as cortisone effected reactivation of influenza virus through mediation by the host cell.<sup>10</sup> Several other problems, basic as well as practical, arise immediately, such as, range of activity as to strain and type of virus, locus of activity, mechanism of action. Speculation is hardly warranted on any of these problems at this time. In view of the data presented, further intensive study on the antiviral action of B139 and related compounds appears to be indicated.

#### CONCLUSIONS AND SUMMARY

A new steroid acid amide of diaminodiphenyl sulfone was synthesized along with other new steroid derivatives and was found to be an active inhibitor of the multiplication of the PR8 strain of influenza virus A in the chick embryo. Virus and drug were injected into the allantoic cavity. Findings on 143 treated eggs and 76 inoculated, nontreated eggs are tabulated. The results show that with massive inocula of about  $10^5$  infective doses, in up to 25 per cent of the treated eggs, the plain allantoic fluids were practically negative for hemagglutinins and 35 per cent showed a titer of less than 1:16. With inocula of about  $10^4$  infective doses, in up to 50 per cent of the treated eggs, the undiluted fluids were negative and 80 per cent had a titer of less than 1:16. With inocula of about  $10^3$  infective doses, up to 40 per cent of the treated fluids were negative and up to 90 per cent had a titer of less than 1:16. Further intensive studies on the antiviral action of B139 and related compounds is indicated.

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I am deeply grateful to Dr. G. K. Hirst for letting me have the influenza virus used in these experiments and to his assistant, Miss Rose Bergamini, for the demonstration of the chick embryo techniques as used in Dr. Hirst's laboratory at the Public Health Institute of the City of New York.

My thanks go to Miss Grace Frank, who has been my collaborator for many years, for her assistance in some of the experiments.

I am much obliged to Dr. F. M. Robinson of Merck Sharp & Dohme who kindly supplied me with a sample of a halogenated ribofuranosyl benzimidazole used as reference drug in a few experiments.

#### BIBLIOGRAPHY

1. BERCZELLER, A.: Steroid acid amides of diamino diphenyl sulfone, *Dis. of Chest* 33:474-481, 1958.
2. HIRST, G. K.: Hemagglutination by viruses. *In: RIVERS, T. M.: Viral and Rickettsial Infections of Man*, Philadelphia, J. B. Lippincott Co., 1952, pp. 100-108.
3. BEVERIDGE, W. I. B., AND BURNETT, F. M.: The Cultivation of Viruses and Rickettsiae in the Chick Embryo, Medical Research Council Special Report Series no. 256, London, Her Majesty's Stationery Office, 1955.
4. HIRST, G. K.: The agglutination of red cells by allantoic fluid of chick embryos infected with influenza virus, *Science* 94:22-23, 1941.
5. SALK, J. E.: A simplified procedure for titrating hemagglutinating capacity of influenza virus and corresponding antibody, *J. Immunol.* 49:87-88, 1944.
6. TAMM, I.; FOLKERS, K.; SHUNK, C. H., AND HORSFALL, F. L., JR.: Inhibition of influenza multiplication by N-glycosides of benzimidazole, *J. Exper. Med.* 99:227-250, 1954.
7. TAMM, I.; FOLKERS, K., AND SHUNK, C. H.: High inhibitory activity of certain halogenated ribofuranosyl benzimidazoles on influenza virus B multiplication, *J. Bact.* 72:54-58, 1956.
8. HOLLINSHEAD, A. C., AND SMITH, P. K.: Effect of certain sulfur-containing compounds on the multiplication of poliomyelitis virus in tissue cultures. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, pp. 927-935.
9. COGGESHALL, L. T., AND MAIER, J.: Effect of various sulfonamides, sulfones and other compounds against experimental influenza and poliomyelitis infections in white mice, *J. Pharmacol. & Exper. Therap.* 76:161-166, 1942.
10. KILBOURNE, E. D.: Reactivation of non-infective virus in a cortisone injected host, *J. Exper. Med.* 101:437-450, 1955.

# Treatment of Amebiasis: Toxicity to Macaques of the Suppressing Drug Dichlorosalicylanilide

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Studies of the toxic effects of antibiotics and other chemotherapeutic agents on the blood of higher animals are still largely confined to examination of peripheral tissues in the dog.<sup>1-3</sup> However, to determine whether potential damage to the hemopoietic system could be detected, we felt it desirable to include biopsy of bone marrow. We also considered the macaque to be a more suitable experimental animal than the dog, as it is more closely related to man. When evaluating new agents for long-term therapy, a clear-cut demonstration of drug effects in a few macaques might be more helpful to clinicians than studies of larger groups of less closely related mammals.

Suppressive drug therapy has proved invaluable in malaria control during and since World War II, but as yet no other protozoan disease has yielded to this type of control. In amebiasis, arsenical drugs, such as carbarsone, were used with partial success by Frick and his associates in Korea.<sup>4,5</sup> Another drug, glycobiarsol, was used in Mississippi with some encouraging results to prevent amebic recurrence by Beaver et al.<sup>6</sup> Iodochlorhydroxyquinoline has been used extensively by laymen, especially outside the United States. Its high iodine content and ready absorption may favor the production of iodism; it is recommended, however, in preference to other available agents.<sup>7</sup>

Kraft<sup>8</sup> reported in 1950 that compounds with double ring structure, especially halogenated salicylanilides, had antibacterial properties. Kraushaar,<sup>9</sup> in 1954, studied the effectiveness of a series of related halogenated salicylanilides, especially against dermatophytes. He also found some activity against *Staphylococcus aureus* and *Escherichia coli* in vitro. When chlorine was the substituent group in both rings, forming 4, 5-dichlorosalicylanilide, the activity was enhanced. The optimum activity was believed to be reached by dihalogenation, especially in consideration of the breadth of the activity spectrum.

Because of the similarity between the effect of halogenation on hydroxyquinoline activity and the increased activity halogenation provides in the salicylanilide structure, it was believed desirable to study 4, 5-dichlorosalicylanilide against *Entamoeba histolytica* in naturally infected monkeys. It has been shown earlier<sup>10</sup> that this compound has amebicidal activity in vitro comparable to that of emetine hydrochloride. Further, in naturally infected macaques, peroral doses given daily for six months cleared all animals of their pathogenic amebae without apparent untoward effects.

The present report is an extension of the earlier study,<sup>10</sup> i.e., to one year of continuous therapy. We were especially interested in possible toxic effects, particularly on the hemopoietic elements of the peripheral tissues and on the bone marrow in the suppressive daily dose range.

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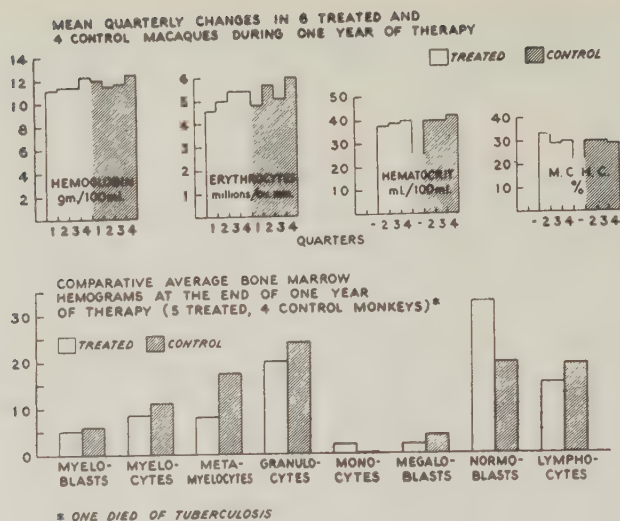


FIG. 1. Results of dichlorosalicylanilide therapy of amebiasis in macaques are given.

## EXPERIMENTAL

Twenty-six monkeys (*Macaca rhesus* or *Macaca philippinensis*) were used in this study. The monkeys were maintained and cared for as has been previously described,<sup>11</sup> with the exception that wheat was substituted for beans. The diet and general care were believed to be adequate, since most of the animals gained and only one lost weight (shortly before death from tuberculosis). All animals were tuberculin-negative before the experiment. There was no evidence of intercurrent disease other than tuberculosis. The group treated for one month was housed by Mr. Marshall Parrott in the Crocker Radiation Laboratory on the Berkeley campus, while animals treated more than one year were housed in our monkey quarters on the San Francisco campus.

Animals were given 4, 5-dichlorosalicylanilide perorally (by "seeding" bananas) seven days a week for the designated length of time, after which survivors were sacrificed for necropsy and histological studies. A number of clinical laboratory tests were carried out, and, since earlier studies in rats and dogs by Seeberg et al<sup>12</sup> had shown occasional abnormal erythrocytes in dogs, our studies in macaques included examinations of peripheral blood. To determine hemopoietic effects not discernible in peripheral blood, punctures of the iliac crests were made. For further substantiation of the results, biopsies of the iliac crest were made under intravenous anesthesia, utilizing pentobarbital, and sections of iliac crest were taken when the animals were sacrificed.

**Experimental Groups.** Six monkeys, naturally infected with *E. histolytica*, were given orally 75 mg./Kg. of the drug daily for a period of one year. A second group of 5 monkeys received daily peroral doses of 100 mg./Kg. for 30 days. Three of these animals harbored *E. histolytica* before therapy, while the other 2 served as uninfected, treated controls.

**Control Groups.** Five monkeys were held for one year under conditions identical with those of the one year experimental group, except that they were given no drug. A second group of 10 freshly imported monkeys was used only for bone marrow studies.

**Results.** All 6 animals in the one year study were cleared of *E. histolytica* in one to four months and remained clear until removed from the experiment by death or sacrifice. Two of them died of tuberculosis, one after eight and the other after 11

months. The 3 infected monkeys in the one month group were also cleared, and there were no deaths in this group. Of the 5 control animals held for one year, 1 died of tuberculosis eight months after the experiment began, 3 were sacrificed in apparent good health at the end of the year, and the fifth (monkey 117) was saved for further study.

No untoward effects of the drug were manifest in any of the 11 treated monkeys, either grossly or in any clinical laboratory test, except examination of blood, bone marrow aspirates, or biopsy material. There was no evident liver damage, since prothrombin time and Bromsulphalein retention were within normal limits. Serum creatinine remained unchanged, indicating adequate kidney function.

Histopathological results were corroborated by Crane, Department of Pathology, University of California Medical Center, while the interpretation of blood and marrow studies were corroborated by members of the Hematology Clinic, University Hospital, San Francisco.

*Blood Studies.* No decrease in leukocytes was observed, except in 1 (tuberculous) animal. As shown in figure 1, there were no significant changes in treated or control groups in hemoglobin, total erythrocyte count, or hematocrit. There was a slight but not significant decrease in mean corpuscular hemoglobin in most of the

TABLE I  
*Clinical-Pathological Changes in Macaques During One Year of Antiamebic Therapy with 4', 5-Dichlorosalicylanilide*

Monkey no.	Weight change, Kg.*	<i>E. histolytica</i>	Morphology of red blood cells	White blood cells	Erythrocyte fragility tests§	Bone marrow changes iliac crest	Remarks
104	+0.4	Cleared	Target cells reticulocytes	0	0.46–0.38	Hyperplastic megakaryocytosis	Died after 1 yr. of therapy gen. tuberculosis
105	+0.4	Cleared	Reticulocytes hypochromia	0	0.48–0.34	Active enchondral ossification	
106	+0.8	Cleared	Hypochromia	0	0.50–0.36	Myelofibrosis advanced	
107	+0.8	Cleared	Target cells	0	0.50–0.36	Myelofibrosis early	
109	+0.3	Cleared	Target cells occ. reticul.	0	0.44–0.32	Myelofibrosis advanced	
113	–1.0†	Cleared	Hypochromia	‡	0.40–0.28	Not studied	Died at end of 9 mo. gen. tuberculosis
112	+0.8	Negative control	Occ. reticulocyte	0	0.46–0.38	Normal	Slight nephrosis
114	+0.1	Negative control	0	0	0.46–0.34	Slight endosteal fibrosis	Mild interstitial pyelonephritis
115	0	Negative control	0	0	0.46–0.40	Active enchondral ossification	
117	+1.0	Negative control	0	0	0.46–0.36	Myelofibrosis moderate	

\* + = gain; – = loss; 0 = no change.  
† Weight loss due to tuberculosis. All monkeys were tuberculin negative before the experiment.  
‡ The white cell count was reduced to 4000 cells/cu. mm.  
§ Average range from 0.48 to 0.40.



FIG. 2. This is the bone marrow of monkey no. 115 (control) obtained by postmortem sectioning of the iliac crest. There is evidence of active enchondral ossification. The marrow is approximately 70 per cent comprised of hemopoietic elements with the usual distribution and 30 per cent fat (X 150).

animals receiving the drug. Our observed hematological values in monkeys correspond closely with those published by Poppen et al.<sup>13</sup> Bleeding and clotting time also fluctuated within average limits.

On the other hand, there was moderate reticulocytosis and target-cell formation in 3 of the 6 monkeys treated for one year (table I), and erythrocyte fragility was slightly decreased in 4 of them.

*Bone Marrow Studies.* When iliac crest punctures were made on the 5 treated survivors in the one year group, there appeared to be an increase in erythroid elements in the marrow. The ratio of myeloid to erythroid cells (M/E) was 1.5:1.0, as compared to a ratio of 2.5:1.0 in the control group. If these findings are contrasted with the results of the peripheral blood studies, the value of bone marrow aspirations is obvious. The iliac crest postmortem sections (table I) showed fibrosis of the bone marrow in 3 of the 5 monkeys, while only 1 of the 4 control monkeys exhibited these changes. This control animal, submitted to iliac crest biopsy at the end of the year, was held for further study. Subsequent biopsy, eight months later, did not show fibrosis.\* To appraise the significance of the latter finding in interpre-

\* At eight months, there was considerable osteoblastic and osteoclastic activity in what appears as normal bone marrow. No fibrosis present *per se* (fig. 5).

tation of the over-all results, similar biopsies were done on 10 healthy, untreated macaques, and normal marrow was found in each case. No abnormalities were found in the marrow of the 5 monkeys treated for one month.

Photomicrographs of normal and damaged bone marrow are reproduced in figures 2 and 3. Figure 2 represents normal marrow of one of the control monkeys, while figure 3 exhibits extensive fibrotic changes in the marrow of one of the monkeys treated for one year. Figure 4 is a section of iliac crest of the control animal that had shown myelofibrosis.

#### POSTMORTEM FINDINGS

*Experimental Group.* (Tissues not abnormal except as noted.) Monkey 104 (died after 11 months of treatment): lungs, focal areas of confluent miliary granuloma with central necrosis, and Langhans giant cells containing *Mycobacterium tuberculosis*; spleen, much enlarged and appeared as a conglomeration of caseating tubercles; and bone marrow (iliac crest), hyperplastic, containing many megakaryocytes.

Monkey 105: bone marrow, active enchondral ossification; and marrow composed

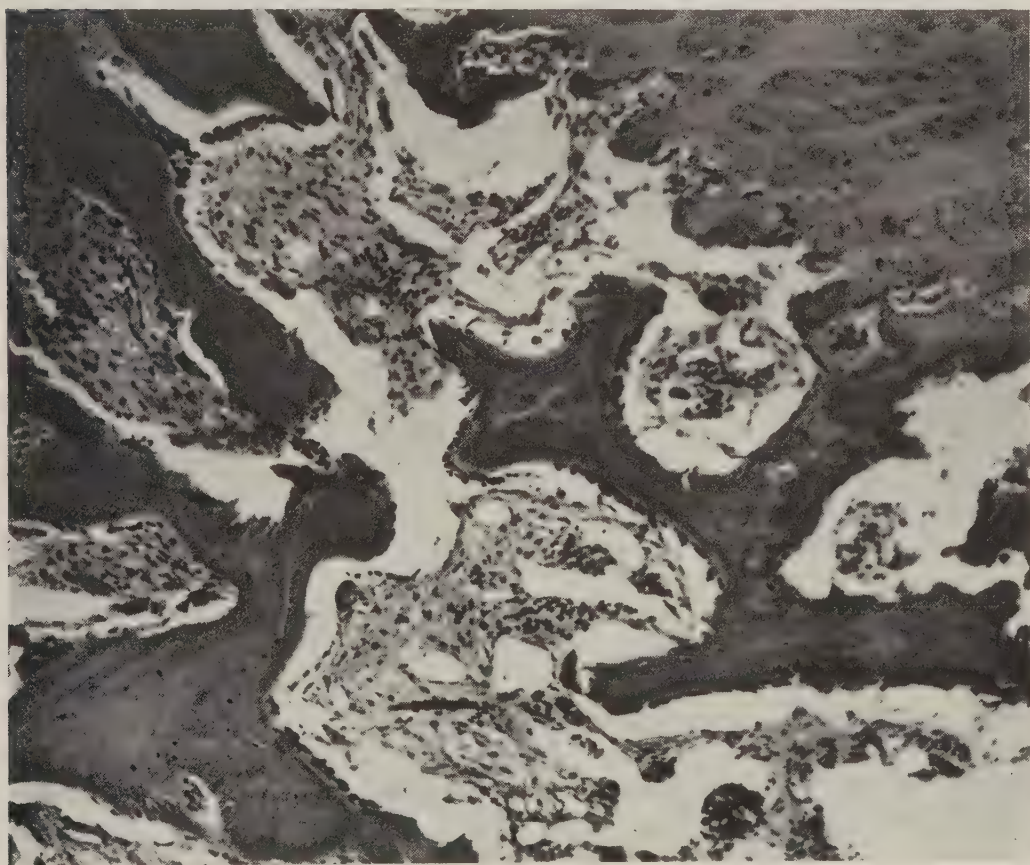


FIG. 3. Shown is the bone marrow of monkey no. 109, treated for one year with dichlorosalicylanilide. The marrow has been replaced with rather vascular, loosely arranged fibrous tissue. There are only a few small islands of myelopoietic elements remaining. This myelofibrosis is associated with increased osteoblastic and osteoclastic activity throughout the metaphysis (X 150).

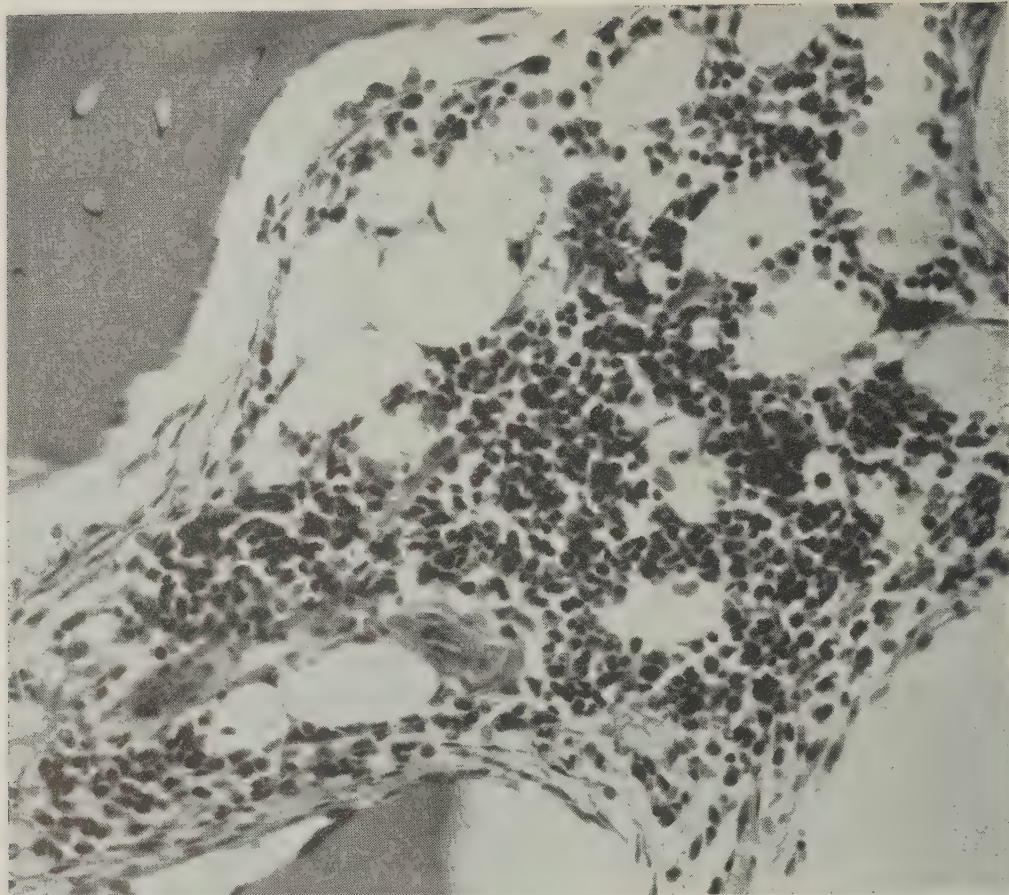


FIG. 4. Pictured is the bone marrow of monkey no. 117 (control), obtained by biopsy of the iliac crest. This section shows patchy areas of cellular marrow with some fibrosis (X 300).

of ratio of two-thirds fat cells and one-third hemopoietic elements, with normal distribution of myelopoietic and erythropoietic cells.

Monkey 106: bone marrow, active enchondral ossification of epiphysis and metaphysis; marrow cavity replaced by vascular, loosely arranged fibrous tissue with a few small focal areas of hemopoietic activity. The iliac crest section was almost devoid of marrow. Sections of rib and sternum were not abnormal except for hyperplasia and numerous megakaryocytes.

Monkey 107: bone marrow, slight, early myelofibrosis, particularly along the endosteum, as well as some osteoblastic and osteoclastic activity. There was a slight shift to the left in the hemopoietic series.

Monkey 109: spleen, some lymphoid hyperplasia; liver, increased periportal fibrous tissue, with disorganization suggestive of mild cirrhosis; bone marrow, replacement of marrow by vascular, loosely arranged fibrous tissue. A few small islands of myelopoietic elements. Increased osteoblastic and osteoclastic activity throughout the metaphysis (fig. 3).

Monkey 113 (died after nine months treatment): general dissemination of tuberculous infection. No study of bone marrow was made.

*Control Group.\** Monkey 112: spleen, increased fibrous strands in septal con-

\* One control died of tuberculosis after eight months; one other (115) revealed no abnormalities.

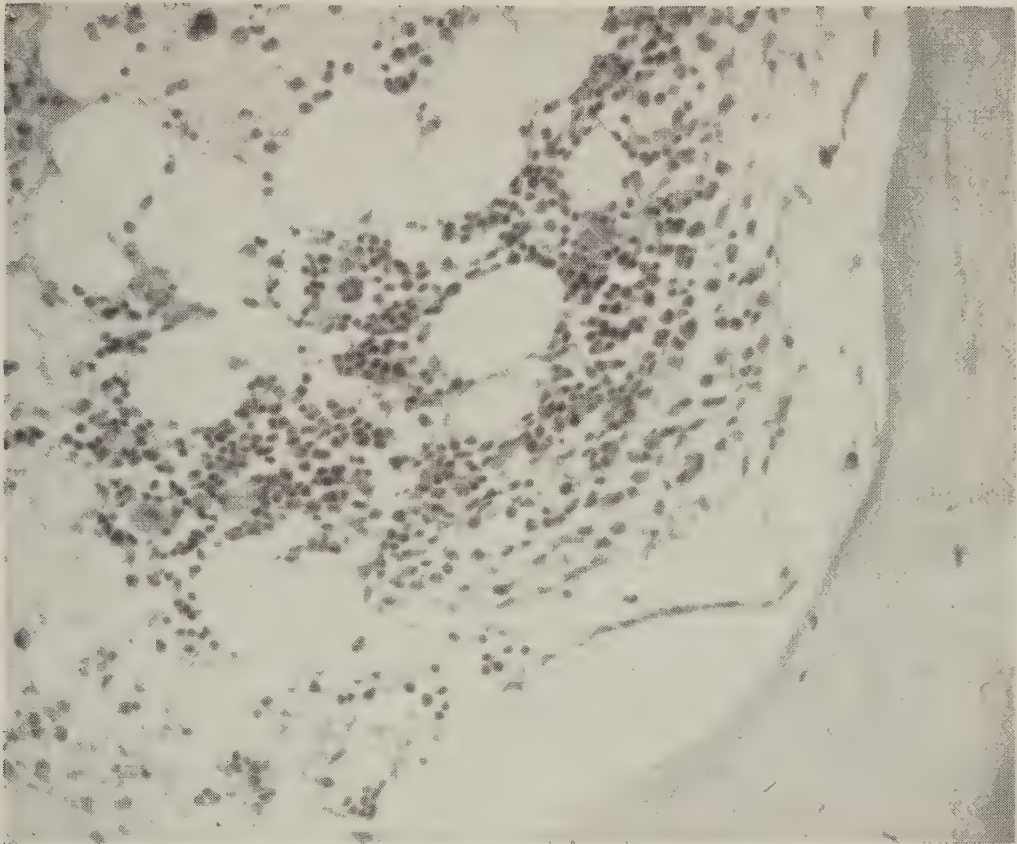


FIG. 5. This is the bone marrow of monkey no. 117 at eight months. There is considerable osteoblastic and osteoclastic activity in what appears as normal bone marrow. There is no fibrosis present *per se* (X 300). Compare with figure 4.

nective tissue; kidneys, suggestive of nephrosis, with dilated convoluted tubules and intraluminal granular eosinophilic elements and fat. Bone marrow, not abnormal.

Monkey 114: large bowel, submucous macrophages containing phagocytized hemosiderin between muscle bundles, within lamina propria; kidneys, mild degree of chronic interstitial pyelonephritis; bone marrow, slight endosteal fibrosis; hemopoietic elements not abnormal.

#### DISCUSSION

Prior to clinical trials of new chemotherapeutic agents, it is highly desirable to establish their safety and possible beneficial effects in laboratory animals. It was felt by the investigators that monkeys would best serve this purpose, since phylogenetically they are more closely related to man than other laboratory animals. If, in addition, the monkeys are closely observed (like patients in a hospital) and daily records are kept, together with clinical-laboratory tests, weight, adequate diet, and so on, the information derived from such observations is invaluable. The risk of encountering "therapeutic accidents" later, when the drug is used for the first time clinically, should be largely circumvented.

This study, involving a new and promising anti-amebic agent, 4,5-dichlorosalicylanilide, was conducted over a period of one year. An abundance of material has been collected, and an attempt has been made to analyze this material critically and present it objectively.

The drug was found to be effective in eliminating and preventing recurrence of *E. histolytica* in naturally infected macaques. Doses of 75 to 100 mg./Kg. were used daily, and the animals tolerated treatment without evidence of untoward effects. The drug was administered by "seeding" bananas, and no apparent distress was noted in any animal.

Mild changes produced in the blood of treated monkeys after many months of ingestion of the drug suggested that 4,5-dichlorosalicylanilide was relatively safe as a chemotherapeutic agent. However, as the chemical structure of this compound would alert one to the possibility of damage to the hemopoietic system not detectable by tests of the peripheral blood, it was mandatory that bone marrow studies be performed. Previous investigators of the drug observed suggestive effects on the blood of dogs;<sup>12</sup> that is, mild anemia and some apparently abnormal erythrocytes. No bone marrow studies were done in these animals.

Selye<sup>16</sup> produced myelofibrosis in rats by injection of the antibiotic puromycin. It is interesting to compare this complex amino nucleoside with the structurally different, unrelated chemical, 4,5-dichlorosalicylanilide, since the former drug has been reported to be effective against experimental trypanosomiasis and has been investigated experimentally against amebiasis.<sup>14,15</sup> It would appear that the dichlorosalicylanilide did not damage the kidneys nor did it produce aldosteronism and edema, as has been observed in experimental puromycin therapy.<sup>16</sup>

Two of the 5 monkeys that we treated for a full year had definite fibrosis of the marrow of the iliac crest, and a third had changes consistent with beginning myelofibrosis. Studies of the sternum and rib marrow of one of these monkeys did not demonstrate fibrosis. The full significance of these findings is not yet apparent; but a drug capable of harming the hemopoietic system after prolonged administration, producing focal fibrosis of the bone marrow, must be critically evaluated. One of the controls exhibited a similar change, which was not present when biopsy was repeated eight months later. However, no changes were found in the marrow of the iliac crest of 5 monkeys treated daily for one month with 100 mg./Kg. of dichlorosalicylanilide, or in that of 10 monkeys freshly arrived from the Philippine Islands. It is obvious that only repeated experiments with biopsy preceding as well as following drug administration, would clarify these findings. Preferably, macaques would be utilized, with suitable controls.

#### SUMMARY

1. Peripheral blood studies in 6 macaques, treated daily *per os* for one year with 75 mg./Kg. of 4,5-dichlorosalicylanilide, did not indicate significant changes in the hemopoietic system or cytotoxicity. There were occasional hypochromia and moderate reticulocytosis and target cell formation.

2. Bone marrow punctures in 3 of 5 surviving monkeys suggested toxic effects of the drug. There was apparently an increased formation of erythroid elements and suppression of myeloid elements of the marrow.

3. Sections of the marrow of the iliac crest were more definitive, showing damage at the site of hemopoiesis. Two of 5 macaques had advanced, while a third had mild myelofibrosis.

4. Only 1 of 15 control monkeys exhibited this change. This monkey was retained, and biopsy eight months later did not show fibrosis.

5. Biopsies showed no changes in the bone marrow of 5 macaques after daily peroral treatment for one month with 100 mg./Kg. of the drug.

6. All of the 6 naturally infected macaques treated for one year and the three treated for one month were cleared of *E. histolytica*, and there was no recurrence of infection.

## BIBLIOGRAPHY

1. REUTNER, T. F.; MAXWELL, R. E.; WESTON, K. E., AND WESTON, J. K.: Chloramphenicol toxicity studies in experimental animals. Part I. The effects of chloramphenicol and various other antibiotics on malnutrition in dogs, with particular reference to the hematopoietic system, *Antib. & Chemo.* 5:679-711, 1955.
2. RADOMSKI, J. L., AND NELSON, A. A.: Toxic effects of chloramphenicol in dogs. Synthetic and fermentation products compared with Aureomycin, *Federation Proc.* 12:358, 1953.
3. NELSON, A. A., AND RADOMSKI, J. L.: Comparative pathological study in dogs of feeding of six broad-spectrum antibiotics, *Antib. & Chemo.* 4:1174-1180, 1954.
4. FRICK, L. P.; WYKOFF, D. E., AND RITCHIE, L. S.: Mass ambulatory treatment of amebiasis with observations on acquisition of new infection in an area of high endemicity. Paper presented at 6th annual meeting of the Am. Soc. of Trop. Med. & Hygiene, Nov. 1, 1957, Philadelphia, Pa.
5. FRICK, L. P.: Study on the use of carbarsone and chiniofon for mass ambulatory therapy of amebiasis. Personal communication, Jan. 23, 1958.
6. BEAVER, P. C.; JUNG, R. C.; SHERMAN, H. J.; READ, T. R., AND ROBINSON, T. A.: Experimental chemoprophylaxis of amebiasis, *Am. J. Trop. Med.* 5:1015-1021, 1956.
7. ANON.: Prophylaxis of amebiasis (Editorial), *J.A.M.A.* 164:505, 1957.
8. KRAFT, K.: Konstitutionell Zusammenhänge auf dem Gebiet bakteriostatisch wirkender Stoffe, *Pharmazie.* 5:257-259, 1950.
9. KRAUSHAAR, A.: Chemotherapeutische Wirksamkeit von Halogenierten Salicylaniliden in Abhängigkeit von der Konstitution, *Arzn. Forsch.* 4:548-557, 1954.
10. ANDERSON, H. H., AND HRENOFF, A. K.: Suppressive amebicidal activity of 4,5-dichlorosalicylanilide in macaques, *Federation Proc.* 16:1574, 1957.
11. HRENOFF, A. K.: Treatment of money amebiasis with Terramycin, *J. Parasitol.* 37:329-330, 1951.
12. SEEBERG, V.: Personal communication. Cutter Laboratories, Berkeley, Calif., 1956-57.
13. POPPEN, K. J.; GREENBERG, L. D., AND RINEHART, J. F.: The blood picture of pyridoxine deficiency in monkey, *Blood* 7:436-444, 1952.
14. HEWITT, R. I.; WALLACE, W. S.; GAMBLE, A. R.; GILL, E. R., AND WILLIAMS, J. H.: Experimental chemotherapy of trypanosomiasis. III. Effect of Achromycin against *Trypanosoma equiperdum* and *Trypanosoma cruzi*, *Am. J. Trop. Med.* 2:254-266, 1953.
15. TAYLOR, J.: Personal communication, 1958.
16. SELYE, H.: Effect of corticoids upon skeletal and renal changes produced by Stylomycin aminonucleoside, *Proc. Soc. Exper. Biol. & Med.* 96:544-547, 1957.

# Chemoprophylaxis of Poliomyelitis in Mice Through the Administration of Plant Extracts

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Recent reviews on the experimental chemotherapy and chemoprophylaxis of virus diseases<sup>1,2</sup> illustrate the diversity of substances studied and the paucity of truly favorable results. Since earlier studies had demonstrated that substances capable of protecting mice and monkeys from poliomyelitis could be obtained from lower fungi, such as species of *Penicillium*,<sup>3-6</sup> it was of interest to determine if higher fungi, recently reported to possess antitumor principles,<sup>7-9</sup> would also contain antiviral substances. The objective in initiating the present investigation therefore was the scrutiny for antiviral activity of different principles possessing known biological action, such as extracts of holobasidiomycetes with anticancer activity, and its scope was expanded to include a variety of green plant materials. This report presents the results to date on the antiviral activity of certain plant extracts against poliomyelitis in mice.

## MATERIALS AND METHODS

Poliomyelitis was produced in young (approximately 10 Gm.) Webster mice by the intraperitoneal inoculation of the MEF<sub>1</sub> strain of type II poliomyelitis virus, using a challenge of approximately 10 ID<sub>50</sub>. These animals were observed regularly for paralysis for 21 days.

Prior to the chemoprophylaxis experiments, all extracts were tested for toxicity, and, if toxic, were diluted to tolerated levels. In testing for chemoprophylactic effectiveness, extracts were given at the rate of 0.02 ml./Gm./day for four days, beginning the day before virus inoculation. Treatment was given by the intraperitoneal route except the second dose, given on the day of virus inoculation, which was injected subcutaneously. This scheme of treatment was designed to minimize non-specific effects that might result from inoculating virus and drug by the same route at the same time. Virus control animals received placebo treatment with equal volumes of buffered saline or 1:10,000 thimerosal. The antiviral activity of each extract was tested at least twice and was evaluated by comparison of the final morbidity in treated and control groups and by a survival index. This index consisted of the ratio of the harmonic mean survival time of the treated to that of the control groups, so that effectiveness in terms of either delaying the onset or decreasing the incidence of disease is reflected by ratios greater than one.

Plant sources were selected on the basis of known or suspected biological properties that suggested a possibility of antiviral activity. Extracts were prepared by treatment of specified plants or parts of plants with water and/or ethanol. Ethanol extracts were evaporated to dryness and used as an aqueous solution or suspension of the residue. Thimerosal (1:10,000) was added to all extracts.

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TABLE I  
*Mushroom Extracts with Antiviral Activity Against Poliomyelitis in Mice*

Number	Source	Dose, ml./Kg./day	Survival index	Morbidity, %	
				Treated	Control
21	<i>Boletus frostii</i>	20	1.5	67	80
38	<i>Calvatia gigantea</i> no. 643	20	1.9	77	97
39	<i>Calvatia gigantea</i> no. 643	20	1.6	83	97
95	<i>Calvatia gigantea</i> no. 713	20	1.3	68	86
40	<i>Lepiota morgani</i>	5	1.3	71	92
23	<i>Russula emetica</i>	20	1.5	82	85
65	<i>Agaricus campestris</i>	20	1.6	73	90
82	<i>Panaeolus subbalteatus</i> no. 684	20	1.7	66	84

Although in all cases certain criteria were present that suggested the application of the plants used, materials were used according to their availability at the time of experimentation. A considerable number of plants that would have been of equal promise on theoretical grounds were omitted in this initial study.

On the basis of experience gained in earlier work dealing with antibacterial properties of plant extracts<sup>10,11</sup> the plants were selected in such a manner that the largest possible number of families and genera was covered. This was done because biologically active substances of similar nature had often been found to occur within families or genera.

## RESULTS

*Preparations of Holobasidiomycetes.* Some overlapping of the two areas of antitumor and antiviral activity is apparent from the extensive study of the action of nucleic acid analogues against viruses<sup>1,2</sup> and has also been demonstrated with certain natural products.<sup>12-15</sup> In view of the presence of antitumor activity in certain mushrooms,<sup>7-9</sup> it was of interest to examine selected species for antiviral activity against poliomyelitis. In the process of studying extracts representing 13 species of Basidiomycetes, eight were found to have a degree of activity represented by survival indices of 1.3 or more. These preparations are listed in table I. The antiviral activity of these preparations against poliomyelitis in mice varied from delay in the onset of paralysis in the case of M23, *Russula emetica*, to the protection seen with M38, *Calvatia gigantea*, strain 643. All were preparations from sporophores except M82, an in vitro surface culture of mycelium. Lack of activity of certain other extracts may be related to culture conditions. Many of the active extracts either contain antitumor activity or are believed to be similar to antitumor extracts.

*Orchidaceous Plant Preparations.* During World War II it was reported that some of the natives of New Guinea used the flowers of certain orchids as drugs for the treatment of some obviously contagious diseases.<sup>16</sup> On this basis, extracts of certain orchids were included in the series to ascertain if the afore-mentioned use reflected the presence of antiviral principles. Of 46 extracts representing 17 strains and species of orchids, 21 were found to provide some degree of protection against poliomyelitis in mice and are listed in table II. The most active fraction was M14, an aqueous solution of the ethanol-extracted residue from the flowers of a species of *Cattleya*. Given at the rate of 20 ml./Kg./day for four days and in the manner described, this material reduced the incidence of paralysis from 88 per cent in the control groups to 50 per cent in the treated groups. Activity was obtained from flowers of several commercial hybrids from which it seems released best but not completely by pro-

TABLE II

*Extracts of Orchidaceous Plants Active Against Poliomyelitis in Mice*

Number	Source	Dose	Survival index	Morbidity	
				Treated	Control
14	<i>Cattleya</i> sp.	20	2.6	50	88
15	<i>Cattleya</i> sp.	20	1.6	59	83
43F1	<i>Cattleya</i> sp.	20	1.5	80	91
43F6	<i>Cattleya</i> sp.	20	1.4	88	90
43F7	<i>Cattleya</i> sp.	10	1.3	77	95
44F1	<i>Cattleya</i> sp.	20	1.6	66	91
47F3	<i>Cattleya</i> sp.	20	1.5	66	84
48F2	<i>Cattleya</i> sp.	20	1.7	65	88
49	<i>Cattleya</i> sp.	20	1.3	66	88
54	<i>Cattleya</i> sp.	20	1.5	62	84
56	<i>Cattleya</i> sp.	20	1.3	81	91
57	<i>Cattleya</i> sp.	20	1.4	83	90
58	<i>Cattleya</i> sp.	20	1.4	88	90
67	<i>Cattleya</i> sp.	20	1.5	75	92
69	<i>Cattleya</i> sp.	20	2.0	63	92
73	<i>Cattleya</i> sp.	20	1.3	81	92
78	<i>Cattleya</i> sp.	10	1.7	59	84
72	<i>Cattleya bowringiana</i>	20	1.4	68	90
30	<i>Paphiopedilum</i> sp.	20	1.7	56	94
52	<i>Miltonia</i> sp.	20	1.3	65	84
53	<i>Miltonia</i> sp.	20	1.5	60	84

longed, mild ethanol extraction. Activity was also obtained from the leaves and pseudobulb of *Cattleya* species by aqueous extraction, and from selected species of related genera.

The characteristics of these and other fractions suggest that the active principle might be either water-soluble pigment precursors or the leuco-form of certain pigments which ethanol removes by dehydration. Although some of the active extracts are highly pigmented, these pigments may not be responsible for antiviral activity since several of the inactive preparations were also highly colored.

*Hypericum Preparations.* Medicinal use of plants from the genus *Hypericum* originated in folklore, and these plants have been shown to contain antibacterial activity.<sup>17</sup> It was of interest therefore to determine if they also contained antiviral activity. As indicated by data in table III, certain species do provide protection against poliomyelitis in mice. Both alcoholic and aqueous extracts of the roots of *Hypericum perforatum* were effective, reducing the incidence of poliomyelitis from 92 per cent in the controls to 66 per cent in the groups treated with M27, and from 94 to 67 per cent in groups receiving M28. Protection was also obtained with aqueous extracts of the flowers of *H. prolificum*.

TABLE III

*Extracts of Hyperica Active Against Poliomyelitis in Mice*

Number.	Source	Dose	Survival index	Morbidity	
				Treated	Control
20	<i>Hypericum</i> sp.	20	1.4	77	85
27	<i>Hypericum perforatum</i>	20	1.4	66	92
28	<i>Hypericum perforatum</i>	10	1.7	67	94
35	<i>Hypericum perforatum</i>	20	1.7	85	95
62	<i>Hypericum prolificum</i>	20	1.5	78	90
63	<i>Hypericum prolificum</i>	20	2.0	65	90
64	<i>Hypericum prolificum</i>	20	2.0	62	90
77	<i>Hypericum prolificum</i>	20	1.4	85	94

TABLE IV  
Miscellaneous Plant Extracts Active Against Poliomyelitis in Mice

Number	Source	Dose ml./Kg./day	Survival index	Morbidity	
				Treated	Control
61	<i>Allium ampeloprasum</i>	20	2.0	64	90
25	<i>Kalmia latifolia</i>	10	1.5	78	85
59	<i>Maclura pomifera</i>	5	2.4	59	90
60	<i>Phellodendron amurense</i>	10	1.7	68	90
84	<i>Medicago sativa</i>	20	1.4	76	85
12	<i>Ribes hirtellum</i>	20	1.3	68	82

A comparison of the antibacterial and antiviral activities of these species is of interest. It was found<sup>17</sup> that preparations of roots and flowers of *H. perforatum* were strongly antibacterial when ethanol was used as solvent; weak activity was indicated in aqueous extracts prepared by boiling. In contrast, alcoholic extracts of dehydrated flowers showed no antiviral activity while water extracted an antiviral principle (M35). In the case of *H. prolificum*, a potent antibacterial substance could be extracted with ethanol, but cold water extraction as well as an aqueous extract made by boiling produced only a mild indication of bacterial inhibition. The fact that the antiviral principle found in these flowers could be found primarily in aqueous extracts speaks against the identity of the antibacterial and the antiviral substance. It is concluded that the antiviral principle in both *Hypericum* species is water-soluble and may have weak antibacterial properties, but that the ethanol-soluble antibacterial substance does not have antiviral activity.

*Miscellaneous Plant Extracts.* In addition to plants of the three groups discussed, a variety of other species were tested and several found to be active as indicated in table IV. Preparation M61, an aqueous extract of the bulb of *Allium ampeloprasum*, reduced the incidence of poliomyelitis from 90 per cent in the control groups to 64 per cent in the treated groups. In this connection and with regard to the possible correlation of antiviral and antitumor activity, a recent report of the antitumor effects of a preparation from *Allium sativum*<sup>18</sup> is of interest. An aqueous extract of the fruit of *Maclura pomifera* reduced the morbidity of poliomyelitis from 90 to 59 per cent, while extracts of the fruit of *Ribes hirtellum*, M12, and of *Phellodendrom amurense*, M60, also provided some protection. M25, from *Kalmia latifolia*, and M84, from *Medicago sativa*, acted to delay the average time of onset of paralysis.

*Inactive Extracts.* Species that provided preparations found to be inactive against poliomyelitis in mice were *Agaricus placomyces*, *Armillaria mellea*, *Calvatia caelata*, *Coprinus micaceus*, *Panaeolus separatus*, *Beta vulgaris*, *Calceolaris crenatifolia*, *Daucus carota*, *Eleocharis dulce*, *Eupatorium purpureum*, *Fragaria vesca*, *Lens esculenta*, *Polygonum persicaria*, *Rubus* sp., *Rumex acetosa*, *Sambucus nigra*. It should be noted that inclusion of a species in this list indicated that antiviral activity was not extracted in detectable concentration from a given material using a given procedure; active principles possibly may have been present but in concentrations too low to be detectable.

#### DISCUSSION

Although extracts of certain plants were found to have varying activities against poliomyelitis in mice, the chemical identities of the various antiviral principles as well as their mode of action are still a matter of conjecture; studies are in progress which are directed toward the isolation and identification of these compounds.

It has been observed that poliomyelitis has tended to become more of a problem as the living standards of a country improved.<sup>19</sup> This has been explained on an epidemiological basis with reference to better sanitation.<sup>20</sup> One may speculate that an additional, contributing factor could be the exclusion of naturally occurring antiviral principles from the diet as foodstuffs become more refined. In this connection it is interesting to note that certain extracts from this same series have been shown to have activity against certain ECHO viruses.<sup>21</sup> Previously Fischer demonstrated that certain plants also possess antiphage activity.<sup>22</sup>

Hurst and Hull,<sup>2</sup> reviewing the chemotherapy of virus diseases, concluded that there was reason for a "restrained optimism" as to "the possibility that chemical substances may ultimately be used to modify the course of diseases caused by the smaller viruses." The results of this study indicate that the resources of nature as well as the synthetic products of the organic chemist are justifiable areas in which to search, and that the apparently widespread distribution of these substances increases the chances for eventual success in this endeavor. It is possible also that buried in folklore or in some of the traditional or native pharmacopoeias lie descriptions of substances capable of modifying diseases caused by the smaller viruses.

#### SUMMARY

Mice were partially protected from infection with intraperitoneally inoculated MEF<sub>1</sub> poliomyelitis by treatment with a variety of plant extracts. Active extracts were obtained from two strains of *Calvatia gigantea* and several other species of higher fungi; from several *Cattleya* hybrids, and some other species of *Orchidaceae*; from *Hypericum perforatum* and *H. prolificum*; and from species of the following genera: *Allium*, *Kalmia*, *Maclura*, *Phellodendron*, *Medicago*, and *Ribes*.

#### BIBLIOGRAPHY

1. MATTHEWS, R. E. F., AND SMITH, J. D.: The Chemotherapy of Viruses. Advances in Virus Research, Editor K. M. Smith and M. A. Lauffer, 3:49-148, New York, Academic Press, 1955.
2. HURST, E. W., AND HULL, R.: The chemotherapy of virus diseases, Pharmacol. Rev. 8:199-263, 1956.
3. POWELL, H. M., AND CULBERTSON, L. G.: Action of an antiviral mold filtrate against MEF<sub>1</sub> poliomyelitis in mice. Proc. Soc. Exper. Biol. & Med. 83:161-163, 1953.
4. SHOPE, R. E.: An antiviral substance from *Penicillium funiculosum*. I. Effect upon infection in mice with swine influenza virus and Columbia SK encephalomyelitis virus, J. Exper. Med. 97:601-625, 1953.
5. COCHRAN, K. W.; BROWN, G. C., AND FRANCIS, T.: Antiviral action of a mold filtrate on experimental poliomyelitis in cynomolgus monkeys. Proc. Soc. Exper. Biol. & Med., 85:104-106, 1954.
6. COCHRAN, K. W., AND FRANCIS, T., JR.: Antiviral action of helenine on experimental poliomyelitis, Proc. Soc. Exper. Biol. & Med. 92:230-232, 1956.
7. LUCAS, E. H.; RINGLER, R. L.; BYERRUM, R. U.; STEVENS, J. A.; CLARKE, D. A., AND STOCK, C. C.: Tumor inhibitors in *Boletus edulis* and other holobasidiomycetes, Antib. & Chemo. 7:1-4, 1957.
8. LUCAS, E. H.; BYERRUM, R. U.; CLARK, D. A.; REILLY, H. C.; STEVENS, J. A., AND STOCK, C. C.: Oncostatic principles in preparations of certain *Calvatia* species. (Paper presented at the Annual Meeting of the Mycological Society of America, 1958.)
9. STEVENS, J. A., AND REILLY, H. C.: The effect of temperature on the in-vitro elaboration of an oncostatic principle by *Calvatia gigantea*. (Paper presented at the Annual Meeting of the Mycological Society of America, 1958.)
10. GOTTSALL, R. Y.; LUCAS, E. H.; LICKFELDT, A., AND ROBERTS, J. M.: The occurrence of antibacterial substances active against mycobacterium tuberculosis in seed plants. J. Clin. Investigation, 28:920-923, 1949.
11. LUCAS, E. H.; LICKFELDT, A.; GOTTSALL, R. Y., AND JENNINGS, J. C.: The occurrence of antibacterial substances in seed plants with special reference to *Mycobacterium tuberculosis*. Bull. Torrey Bot. Club 78:310-321, 1951. Also: Michigan Agric. Exper. Sta. Quart. Bull. 36:477-488, 1954; 37:425-436, 1955; 38:52-59; 60-66; 279-287, 1955.

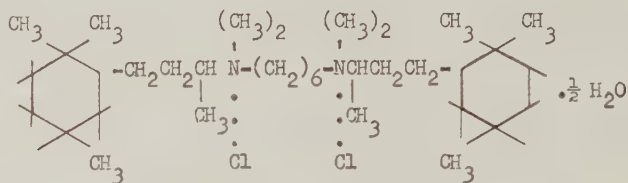
12. JOHNSON, I. S., AND BAKER, L. A.: The utility of the Rous sarcoma virus for the detection of antiviral activity, *Antib. & Chemo.* 8:113-121, 1958.
13. JOHNSON, I. S.: Further studies on the utility of the Rous sarcoma virus for the detection of antiviral activity. *In: Antibiotics Annual, 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 967-971.
14. UTECH, N. M., AND JOHNSON, J.: The inactivation of plant viruses by substances obtained from bacteria and fungi, *Phytopathology* 40:247-265, 1950.
15. MITCHELL, J. W.: Personal communication to E. H. Lucas, 1956.
16. ANON.: Personal communication to E. H. Lucas through correspondence with students in military service, 1945.
17. FRISBEY, A.; ROBERTS, J. M.; JENNINGS, J. C.; GOTTSALL, R. Y., AND LUCAS, E. H.: The occurrence of antibacterial substances in seed plants with special reference to *Mycobacterium tuberculosis*, *Michigan Agric. Exper. Sta. Quart. Bull.* 35:392-404, 1953.
18. WEISBERGER, A. S., AND PENSKEY, J.: Tumor inhibiting effects derived from an active principle of garlic (*Allium sativum*), *Science* 126:1112-1114, 1957.
19. PAYNE, A. M. M.: Poliomyelitis as a world problem. *In: Poliomyelitis; Papers and discussion presented at the Third International Poliomyelitis Conference.* Philadelphia, J. B. Lippincott Co., 1955.
20. GEAR, J. H. S.: Distribution of antibodies to poliomyelitis virus in the general population. *In: Poliomyelitis; Papers and discussion presented at the Third International Poliomyelitis Conference.* Philadelphia, J. B. Lippincott Co., 1955.
21. GOULET, N. R.: Doctoral dissertation: Differential chemical inhibition of poliomyelitis, coxsackie, vaccinia, and ECHO virus in HeLa, monkey kidney, and monkey testicular tissue cultures. University of Michigan, 1958.
22. FISCHER, G.: Presence de substance antivirotiques dans differentes parties de plants. *Ann. Inst. Pasteur* 82:119, 1952.

# Clinical Appraisal of a New Topical Quaternary Compound, Ro 5-0810/1

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Ro 5-0810/1 (Triburon\*) is a new bisquaternary diamine derived from beta-ionone. Chemically, it is N,N'-bis[1-methyl-3-(2,2,6-trimethylcyclohexyl) propyl]-N,N'-dimethyl-1,6-hexanediamine bis (methochloride) hemihydrate with a structural formula as shown.<sup>1</sup>



Laboratory studies have shown that it is of moderate toxicity. Its potential as a topical antibacterial is based on the fact that: (1) it exerts a high bacteriostatic and a moderate bactericidal activity against both gram-positive and gram-negative organisms in vitro, and (2) it has marked local antibacterial effect against *Streptococcus hemolyticus* and *Micrococcus pyogenes* var. *aureus* in vivo regardless of their resistance to antibiotics.<sup>2</sup>

This report gives the results of our clinical experiences with Ro 5-0810/1. It includes an evaluation of skin tolerance as well as observations on the drug's activity in the treatment of bacterial infections of the skin.

## IRRITATION AND SENSITIZATION TESTS

Ro 5-0810/1 was supplied as a 0.1 per cent ointment in Carbowax and vanishing cream bases. Initial irritation and sensitization studies were started on 220 volunteers utilizing the "multiple closed patch technique" developed by Draize, Chief of the Skin and Toxicity Branch of the Food and Drug Administration. Ten closed patches, each containing 0.5 Gm. of ointment, and the ointment bases alone as controls were applied in a systematized fashion to the forearms, arms, or back of each volunteer. The interval between applications was 48 hours. The areas under each patch were examined on their removal, and estimates of the amount of erythema and edema were made. After this series of 10 applications of each ointment, the subjects were given 14 days' rest. A challenge or retest dose (0.5 Gm. of ointment in a patch) was then applied. This was read at 48 and again at 72 hours.

Of the 220 patients started initially in this investigation, 209 completed the 10 patches of the two ointment preparations and the two control ointment bases. The 11 persons who discontinued the testing in various stages did so for reasons unrelated to the study, including respiratory infections, adhesive tape irritations, and various personal reasons that prevented them from reporting at the regular intervals as was necessary for the proper execution of these experiments. Four of these volunteers, less than 2 per cent, developed allergic reactions to Ro 5-0810/1. These four reactions appeared as responses to the final challenge dose, which was the end result of the series of 4598 applications of Ro 5-0810/1 in either the Carbowax or vanishing cream base. Three persons developed an allergic response to the Carbowax

\* The trade name of Hoffmann-La Roche, Inc., for Ro 5-0810/1 is Triburon.

TABLE I  
Clinical Response to Ro 5-0810/1\*

	No.	Clinically cured in days										Cured	Mark- edly im- proved	Moder- ately im- proved	Unim- proved
		4	7	10	14	17	21	24	28	32					
Primary Infection															
Impetigo contagiosa	42	3	6	9	5	2	2	1	—	—	28	9	3	2	
Sycosis vulgaris	8	—	—	—	1	2	—	—	—	—	3	3	2		
Folliculitis, acute	11	1	1	3	1	1	—	—	—	—	7	2	2		
Furunculosis, acute	3	—	—	—	1	—	—	—	—	—	1		1	1	
Furunculosis, chronic	4	—	—	—	—	—	—	—	—	—	0	1	1	2	
Folliculitis keloidalis	2	—	—	—	—	—	—	—	—	—	0	1	1		
Infectious eczematous dermatitis	5	—	—	—	—	—	—	—	—	—	3†	1	1		
Secondary Infection															
Tinea capitis	7	—	1	—	—	—	1	—	1	1	4			3	
Varicose ulcers	3	—	—	—	1	—	1	—	—	—	2	1			
Seborrheic dermatitis	5	—	1	2	—	—	1	—	—	—	4	1			
Erythema multiforme	1	—	—	1	—	—	—	—	—	—	1				
Dermatitis factitia	1	—	—	—	1	—	—	—	—	—	1				
Lupus erythematosus	2	—	—	1	1	—	—	—	—	—	2				
Pediculosis pubis	1	—	—	1	—	—	—	—	—	—	1				
Intertrigo	2	—	—	—	—	—	—	—	—	—	0	1		1	
Excoriation (neurotic)	1	—	—	—	—	—	—	—	—	—	0	1			
Contact dermatitis	1	—	—	1	—	—	—	—	—	—	1				
Dermatitis venenata	5	—	1	1	—	—	—	—	—	—	2	2	1		
Dermatophytosis	3	—	—	1	—	1	—	—	—	—	2		1		
Perlèche	1	—	1	—	—	—	—	—	—	—	1				
Paronychia	2	—	—	—	1	—	—	—	—	—	1	1			
Miscellaneous Infection															
Hydradenitis suppurativa	2	—	—	2	—	—	—	—	—	—	2				
Granuloma pyogenica	1	—	—	—	—	—	—	—	—	—		1			
Total	113	—	—	—	—	—	—	—	—	—	66	25	13	9	
Per cent											58.4	22.5	11.5	7.6	

\* Subsequent to the submission of the original paper an additional number of patients have been treated bringing the total to 161 patients. Of these 98 were cured (61 per cent), 34 were markedly improved (21 per cent), 17 were moderately improved (10 per cent), and 12 were unimproved (8 per cent).

† A cure is much more gradual in eczematous conditions than in most other dermatidides and therefore no specific end point could be fixed as to the cure time. However, these 3 patients averaged approximately 24 days of treatment before a "cure" was considered to be obtained.

base alone. And only 1 person developed an allergic response to the vanishing cream base.

#### CLINICAL EFFECTIVENESS

After the completion of the study of sensitization and irritation by the closed patch technique, 113 patients with a variety of bacterial skin infections, both primary and secondary in character, were treated with either boric acid aluminum subacetate solution compresses followed by light applications of Ro 5-0810/1 in a Carbowax base. These patients were instructed to rub the ointment lightly into the affected

TABLE II  
Organisms Cultured from 30 Cases

	Before treatment	After treatment
<i>Str. hemolyticus</i>	12	1
<i>M. pyogenes</i> var. <i>aureus</i>	25	4
<i>E. coli</i>	2	0
	39	5

TABLE III  
Bacteriological Failures

	Cured	Improved	Failures
<i>Str. hemolyticus</i>	—	—	1
<i>M. pyogenes</i> var. <i>aureus</i>	2	1	1

area three times a day. No adjunctive therapy, oral or parenteral, was employed during the period of evaluation.

Bacteriological cultures were taken from one or more representative lesions in 30 selected cases before instituting therapy and at the end of the observation period. Observations of the patients were at a semiweekly interval and the maximum evaluation period was 32 days.

The type of bacterial infections seen and the clinical responses are summarized in table I. Of the total of 113 patients observed, 66 or 58.4 per cent were classified as clinically cured and this response occurred in the majority of persons in from one to three weeks. Twenty-five or 22.5 per cent of the patients showed marked improvement. Thirteen or 11.5 per cent were moderately improved and 9 or 7.6 per cent were unimproved.

Thirty-nine organisms were isolated from the 30 patients from whom cultures were initially taken. These are tabulated in table II and included *Str. hemolyticus*, 12 strains; *M. pyogenes* var. *aureus*, 25 strains; and *Escherichia coli*, 2 strains. At the end of the observation period, 5 patients were still bacteriologically positive. Of these, 2 were classified as clinical failures, 1 had shown marked improvement, and 2 were recorded as clinically cured. In 2 of these 5 cases (1 was classified clinically as markedly improved and 1 a failure), although both *Str. hemolyticus* and *M. pyogenes* var. *aureus* had been initially isolated, only *M. pyogenes* was found on final culture. Of the remaining 3 patients, *M. pyogenes* var. *aureus* was isolated from 2, both clinically cured, and *Str. hemolyticus* from the other (a clinical failure). Thus, the bacteriological cure rate may be considered to be between 84 and 90 per cent (table III).

No signs of irritation or sensitization were observed in any of the cases studied.

#### CONCLUSIONS

In a series of 113 patients with various bacteriological infections treated with 0.1 per cent Ro 5-0810/1 in a Carbowax ointment, 91 (84.9 per cent) were classified as cured or markedly improved, 11.5 per cent (13 patients) were classified as moderately improved, and 7.6 per cent (9 patients) were unimproved. Bacteriological studies before and after treatment in 30 patients showed a response rate of 84 to 90 per cent. No evidence of irritation or sensitization was encountered during the clinical trial, and less than 2 per cent allergic response was obtained by the closed patch technique in 209 volunteers who were subjected to 4598 applications of the ointment in both Carbowax and in vanishing cream bases. Ro 5-0810/1 ointment is considered to be safe and effective in the topical treatment of bacterial skin infections.

#### BIBLIOGRAPHY

1. TEITEL, S., AND GOLDBERG, M. W.: Personal communication.
2. SCHNITZER, R. J.; GRUNBERG, E.; DE LORENZO, W. F., AND BAGDON, R. E.: An antimicrobial agent with local activity in living host tissues, Proc. Soc. Exper. Biol. & Med. Submitted for publication.

# Local Application of Triclobisonium Chloride in the Treatment of Pyogenic Dermatoses

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There are two types of organic, nitrogen-containing compounds referred to as quaternary ammonium salts. These result either from replacement of the four hydrogen atoms of the ammonium radical by alkyl or aryl groups, or by alkylation or arylation of the nitrogen of heterocyclic radicals. Bisquaternary compounds are formed by the union of two such ammonium groups. Some of the quaternary ammonium compounds possess antiseptic qualities (e.g., benzalkonium) while others are recommended in commercially available preparations as having anti-fungal properties.

A recently synthesized bisquaternary compound,<sup>1</sup> triclobisonium chloride,\* was found to have in vitro antibacterial properties<sup>2</sup> and was made available in ointment form in a concentration of 0.1 per cent for clinical trial. Two hundred forty-eight patients with pyogenic dermatoses or superficial mycoses have been treated with local applications of this ointment, alone or in combination with hydrocortisone. The results are summarized in the present communication.

## THE STUDY

*Patient Material.* Patients included in this study were from the authors' private practices and the outpatient departments of the Woman's Hospital and the University Hospital. The only criterion for inclusion in the study was the presence of a pyogenic dermatosis. Age, sex, race, and duration of infection had no influence on the treatment results.

*Materials Used.* Triclobisonium chloride, 0.1 per cent, was incorporated in both a vanishing cream base and a polyethylene glycol base. In secondarily infected eczematous eruptions an ointment containing 0.1 per cent triclobisonium chloride and 0.5 per cent hydrocortisone in a polyethylene glycol base was used.

*Method of Study.* Patients were instructed to apply the ointment sparingly, three times daily, to all affected areas. Whenever possible, results of therapy were noted by two or more competent observers.

A preliminary survey to determine primary irritation or local sensitizing effects was made before the study of therapeutic potential was undertaken.

*Results of the Study.* In order to determine whether triclobisonium chloride had primary irritating effect or sensitizing qualities, 132 patients were patch tested. Sixty-six patients had patch tests of the drug in a vanishing cream base applied to the right shoulder, and 66 patients had patch tests of the drug in polyethylene glycol base applied to the right shoulder. These tests were removed in 48 hours and observed for erythema or other local reaction. Twenty-one days after the initial test application, identical patch tests were applied over the same areas in 100 patients. These tests were left in place for 24 hours and the areas then observed for inflam-

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This study was supported by a grant-in-aid from the Hoffmann-La Roche Company.

\* The trade name of Hoffmann-La Roche for triclobisonium chloride is Triburon.

TABLE I

*One Hundred Thirty-two Patients Patch-Tested with 0.1 Per Cent Triclobisonium Chloride in Vanishing Cream Base or Polyethylene Glycol Base and Re-tested in 21 Days*

	Patients with positive patch tests	Second test	
		Total patients*	Positive tests
Vanishing cream base	0	48	0
Polyethylene glycol base	0	52	0

\* Thirty-two patients were lost to follow-up during the three week interval between tests.

matory reactions. It was felt that the elapsed three weeks would be a sufficient incubation period for the second patch test to be an eliciting dose, cutaneous sensitization being manifested by erythema or vesicle formation. The results of these observations are summarized in table I.

The results of these tests indicated that triclobisonium chloride was not a primary irritant, and probably was not a potent sensitizer, when incorporated in concentration of 0.1 per cent in vanishing cream or polyethylene glycol base. Two hundred forty-eight patients with pyogenic dermatoses were treated with triclobisonium chloride 0.1 per cent in polyethylene glycol ointment base. The relative efficacy of triclobisonium chloride, when compared with currently available antibiotic ointments, was determined by treating 38 patients with widespread impetigo contagiosa by the paired-comparison method. Lesions on one extremity or side were treated with triclobisonium chloride ointment, and lesions on the other side with an antibiotic ointment. The antibiotic ointments selected for this part of the study were commercially available combinations containing neomycin and gramicidin in an oleaginous ointment base, and a combination of neomycin, bacitracin, and polymyxin B in an oleaginous ointment base. There was no appreciable difference in therapeutic efficacy of the three ointments, involution in all cases beginning within 24 hours, with complete healing in five days.

Bacterial cultural studies were done on 42 patients with impetigo contagiosa, ecthyma, or dermatitis repens. The results are summarized in table II.

*Staphylococcus aureus* and *Streptococcus pyogenes* were the most frequently cultured organisms in impetigo contagiosa and ecthyma, and *Staph. aureus* and *Staphylococcus albus* were most frequently cultured from lesions of dermatitis repens.

Since triclobisonium chloride had been shown to be effective in the paired-comparison study of 38 patients, 210 additional patients with various pyodermas were treated with the 0.1 per cent ointment, alone or in combination with 0.5 per cent hydrocortisone. The results are summarized in table III.

Sixteen patients with pustular folliculitis were treated for one to three weeks. The pustular element responded well to triclobisonium chloride, but new pustules ap-

TABLE II

*Cultural Studies on Impetigo Contagiosa, Ecthyma, and Dermatitis Repens*

Diagnosis	Number of patients	Cultured organisms
Impetigo contagiosa	12	<i>Staphylococcus aureus</i> <i>Streptococcus pyogenes</i>
Ecthyma	12	<i>Staphylococcus aureus</i> <i>Streptococcus pyogenes</i>
Dermatitis repens	18	<i>Staphylococcus aureus</i> <i>Staphylococcus albus</i>

TABLE III

Two Hundred Ten Patients with Various Pyodermas Treated with Local Applications of 0.1 Per Cent Triclobisonium Alone or in Combination with 0.5 Per Cent Hydrocortisone, in Polyethylene Glycol Ointment Base

Diagnosis	Number of patients	Preparation		Duration of treatment	Results of treatment	Adverse reactions
		Plain	Hydrocortisone combination			
Impetigo contagiosa	50	50		3-10 days	Excellent	None
Ecthyma	24	24		5-14 days	Excellent	None
Pustular folliculitis	16	16		1-3 weeks	Infection controlled	3
Dermatitis repens	36	18		1-2 weeks	Infection controlled	None
Secondarily infected eczematous eruptions	84	20	18	1-4 weeks	Excellent	None
				1-2 weeks	Infection Controlled	None
			64	1-4 weeks	Excellent	1

peared as soon as therapy was discontinued. Thirty-eight patients with secondarily infected eczematous conditions or dermatitis repens were treated with the ointment, the infection responding rapidly, although no effect was noted on the eczematous process. Three patients in this series reacted adversely, complaining that the ointment burned when applied.

Eighty-two patients with dermatitis repens or secondarily infected eczematous eruptions were treated with an ointment containing 0.1 per cent triclobisonium chloride and 0.5 per cent hydrocortisone in a polyethylene glycol base. Only one patient complained that this preparation seemed to be irritating. Seventy-eight of the 82 patients obtained an excellent response, improvement being noted within 24 hours and maintained as long as the ointment was used. As with other locally applied steroid medications, this preparation served only to control symptoms, the eczematous eruption tending to flare when the applications were discontinued.

Since in vitro studies had indicated that triclobisonium had fungicidal or fungistatic value, 21 patients with *Microsporum audouini* tinea capitis, 2 patients with *Microsporum canis* tinea capitis, 3 patients with *Trichophyton gypseum* tinea pedis, 4 cases of *Candida albicans* paronychia, and 2 cases of *Trichophyton rubrum* tinea corporis were treated from 2 to 12 weeks. Three patients with tinea capitis were benefited. There was no appreciable benefit in other patients.

#### SUMMARY AND CONCLUSIONS

1. Triclobisonium chloride was shown to be nonirritating in 48 hour closed patch tests on 132 patients and was not sensitizing in repeat exposures to 100 of these patients after three weeks.

2. Two hundred forty-eight patients with pyogenic dermatoses (bacterial infections of the skin) were treated with local applications of 0.1 per cent triclobisonium ointment. Results show that the drug is effective in the treatment of these conditions.

3. A combination of 0.1 per cent triclobisonium chloride and 0.5 per cent hydrocortisone in polyethylene glycol ointment base is effective in the treatment of dermatitis repens and secondarily infected eczematous eruptions.

4. Triclobisonium chloride, in concentration of 0.1 per cent, was not effective, in this study, in the treatment of superficial mycoses, when applied over periods of 2 to 12 weeks.

5. The therapeutic effectiveness of triclobisonium chloride ointment in the treatment of pyogenic dermatoses is comparable to that obtained with local antibiotic preparations now available.

#### BIBLIOGRAPHY

1. TEITEL, S., AND GOLDBERG, M. W.: Personal communication.
2. SCHNITZER, R. J.; GRUNBERG, E.; DELORANZO, W. F., AND BAGDON, R. C.: Triburon, an antimicrobial agent with local activity in living host tissues, *Proc. Soc. Exper. Biol. & Med.* Submitted for publication.

# Glucosamine Treatment of *Schistosoma mansoni*

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Three species of schistosomes are responsible for serious human diseases: *Schistosoma mansoni*, intestinal schistosomiasis; *S. haematobium*, chiefly urinary schistosomiasis or bilharziasis; and *S. japonicum*, Asiatic intestinal schistosomiasis. The geographical distribution of *S. mansoni* infections is: Africa, West Indies, and South America; the geographical distribution of *S. haematobium* is: Africa, southern Europe, western Asia, and Australia; and the geographical distribution of *S. japonicum* is China, Japan, Formosa, Philippines, and the Celebes.<sup>1</sup>

Schistosomiasis is a world health problem. Stoll<sup>2</sup> estimated in 1946 that the incidence of this parasitism was: *S. mansoni*, 29.2 million; *S. haematobium*, 39.2 million; and *S. japonicum*, 46 million; the total incidence was 114.4 million.

Until recently, relatively few persons with schistosomiasis lived in the continental United States, and, consequently, study of the disease in this country generally has been restricted to the laboratory investigation of experimental infections. The incoming Puerto Rican population of the New York City area has brought with it a number of individuals infected with *S. mansoni*. Indeed, there are now so many cases of *S. mansoni* that the Department of Health of New York City has established a separate schistosomiasis clinic. Fortunately, schistosomiasis is not known to be contagious within the confines of the United States. The latter fact, however, does not discount the seriousness of the disease. Actually, since new infections are not acquired in the New York City area, an unusual condition exists with regard to evaluating the effectiveness of therapy of schistosomiasis.

Because of increasing contact with patients suffering from schistosomiasis, many physicians already have found that the therapy of this parasitism is far from satisfactory. Certain of the trivalent antimonials, particularly potassium antimony tartrate (tartar emetic), and sodium antimony tartrate, frequently are accompanied by severe adverse reactions. Stibophen and anthiomaline, also trivalent antimonials, are less toxic than either of the former, but they are less effective. The pentavalent antimony compounds generally are ineffective. Miracil, a thioxanthone derivative, and its analogues are moderately effective, but their administration usually causes rather severe adverse reactions. Generally speaking, it may be said that those drugs that are less toxic are usually less effective, and even after apparent cures, relapses are common.

A report by Bueding et al<sup>3</sup> on the effects of glucosamine on *S. mansoni* infections in mice stimulated our interest in investigating its use as a therapeutic agent in *Schistosoma mansoni* in human beings. As a result of their studies, Bueding et al supposed that glucosamine (2-amino-d-glucose) interfered with the internal carbohydrate metabolism of *S. mansoni*. We are not aware of any previous reports of studies on the use of glucosamine as a therapeutic agent in human cases of schistosomiasis. Glucosamine, although present in minute quantities in various tissues and body fluids, is known to be a normal human metabolite.<sup>4</sup> Doses of 50 Gm./day have been administered without causing any adverse effects.

Eighteen Puerto Ricans, 7 of whom were children, with *S. mansoni* were treated in the outpatient department with glucosamine. The clinical diagnosis was confirmed by finding the ova of *S. mansoni* in biopsies of the middle rectal valve and in the feces.<sup>5</sup>

The patients were separated into three groups. In group I, there were 4 adults and 3 children who had been treated previously with stibophen or with one of the Miracil analogues and who had relapsed clinically and by rectal biopsy. In group II, there were 4 adults and 3 children who had not been treated previously and thus were considered as "fresh cases." In group III, there were 3 adults and 1 child who were added later in order to evaluate therapy with larger doses of glucosamine when supplies were plentiful; some of these patients had been treated previously.

#### DOSAGE OF GLUCOSAMINE IN SCHISTOSOMIASIS

The glucosamine was administered orally to all of the patients as the hydrochloride salt, at first in 250 mg. capsules and later in bulk, measured by teaspoon, dissolved in coffee or mixed with cereal. The doses, which were discretionary, ranged from 3 to 12 Gm./day given during periods of 10 to 21 days. Earlier in the study, the shorter dosages were determined by the availability of glucosamine.

#### THERAPEUTIC RESULTS

The results of treatment of *Schistosoma mansoni* with glucosamine were evaluated according to post-treatment rectal valve biopsies, which were reported as positive or negative, depending on the presence of the ova of *S. mansoni*. The rectal valve

TABLE I  
Study Results

Group	Glucosamine dosage, Gm./day	Duration, days	Response		Good responses, weeks of treatment*								Patient
			Poor	Good	1	2	3	4	5	6	7	8	
Group I.	Previous treatment												
4 adults	4	10	1	3	0	0	0	0					1
					0	0	0						2
					0	0							3
3 children†	3	10	1	1	0	0							4
Group II.	No previous treatment												
4 adults	4	7	0	4	0	0	0			0	0		5
	then	then			0	0	0			0	0		6
	12	14			0	0	0			0	0		7
					0	0	0			0	x		8
3 children	4	21	0	3	0	0	0				x		9
					0	0	0				x		10
					0	0	x						11
Group III.	Unclassified												
3 adults†	12	21	0	2	0		0						12
					0		0						13
1 child	8	21	0	1	0		0						14
Total			2	14									

\* 0 = negative biopsies; x = positive biopsies.

† Patient did not return for follow-up.

biopsies usually were done at intervals of one or two weeks, beginning on the eleventh day (first post-treatment day in the early part of the study) and continuing currently until eight weeks post-treatment.

*Group I.* Four adults received 4 Gm. of glucosamine daily for 10 consecutive days. The rectal biopsies of 1 patient continued to be negative for the ova of *S. mansoni* for five weeks post-treatment; the rectal biopsies of 2 other patients continued to be negative for four and three weeks, respectively. The rectal biopsies of a fourth patient remained positive, although the number of eggs in the specimen were markedly reduced.

Three children were treated with 3 Gm. of glucosamine daily for 10 consecutive days. One child failed to return for post-treatment examinations. The rectal biopsies of the other 2 children continued to be negative during four weeks post-treatment, after which they became positive for the ova of *S. mansoni*.

It should be noted at this point that these dosages, which we consider as inadequate, were determined chiefly by the short supplies of glucosamine.

*Group II.* No previous treatment for schistosomiasis had been given. The rectal biopsies of 3 of 4 adults, who were given 4 Gm. of glucosamine daily for seven days (during period of short supplies) and then were given 12 Gm. daily for the next 14 days, continued to be negative for ova of *S. mansoni* during eight weeks post-treatment; the rectal biopsies of the fourth patient, having been negative during seven weeks, became positive from the eighth week post-treatment. The rectal biopsies of 2 of 3 children, who were given 4 Gm. of glucosamine daily for 21 consecutive days, were negative through the fourth week post-treatment, while the rectal biopsy of the third child became positive at that time. At eight weeks post-treatment, the rectal biopsies of all of the children were positive for the ova of *S. mansoni*.

*Group III.* The rectal biopsies of 2 of 3 adults, who had received 12 Gm. daily for 21 consecutive days, currently have continued negative for the ova of *S. mansoni* during four weeks post-treatment; the third patient failed to return for post-treatment examinations.

The rectal biopsies of 1 child, who had received 8 Gm. of glucosamine daily for 21 consecutive days, also continued to be negative during four weeks post-treatment.

Although all patients had clinical manifestations characteristic of this parasitism before therapy with glucosamine was begun, these symptoms uniformly disappeared with this treatment and did not recur despite the recurrence of rectal biopsies positive for ova of *S. mansoni*.

*Toxic Reactions.* No immediate or delayed adverse reactions were observed in any of the patients who were treated with glucosamine.

#### DISCUSSION AND CONCLUSIONS

An analysis of these results of treatment in these cases of *S. mansoni* with glucosamine suggests the following.

1. Glucosamine effectively interferes with the egg laying capacity of *S. mansoni* and/or their deposition in the veins of the rectum, thus implying that this phenomenon is caused by a disturbance of the metabolism of the worms and their return to the liver.
2. Glucosamine is remarkably effective in causing a clinical remission of symptoms in *S. mansoni*.

3. The effectiveness of glucosamine in *S. mansoni* infections apparently is related directly to the size and duration of dosage of this amino sugar.

4. The discretionary dosage of glucosamine that we used to treat *S. mansoni* is definitely not the optimal dosage.

5. The treatment of *S. mansoni* with glucosamine is essentially harmless.

6. It is possible that glucosamine might be used alone in the treatment of *S. mansoni* providing it is administered in optimal dosages. If glucosamine is found to be inadequate when given alone, and the early beneficial effects of its administration are not maintained, it could be used as an adjunctive before and/or during therapy with another antischistosomal drug.

7. If the early effectiveness of larger dosages of glucosamine were not maintained, the dosage of this amino sugar for extended periods to maintain negative rectal biopsies and a state of general well-being should be considered—similar to the extended use of liver extract or vitamin B<sub>12</sub> in pernicious anemia. This course of therapy might be feasible in view of the apparent lack of toxicity of glucosamine.

8. Since glucosamine has been shown to be effective against human infections with *S. mansoni*, clinical trials with it in the treatment of *S. haematobium* and *S. japonicum* infections would be indicated.

#### BIBLIOGRAPHY

1. FAUST, E. C., AND RUSSELL, P. F.: Clinical Parasitology, ed. 6, Philadelphia, Lea & Febiger, 1957.
2. STOLL, N. R.: This wormy world, J. Parasitol. 33:1-18, 1947.
3. BUEDING, E.; RUPPENDER, H., AND MACKINNON, J.: Glucosamine kinase of *Schistosoma mansoni*, Proc. Nat. Acad. Sc. 40:773-777, 1954.
4. GITTINGER, W. C., AND BOYER, D. D.: The biologic significance of D-glucosamine, Clin. Rev. 1:9-12, 1958.
5. LOUGHLIN, E. H., AND SPITZ, S. H.: Diagnosis of helminthiasis, J.A.M.A. 139:997-1000, 1949.

# Comparison of Toleration and Absorption of Phenyl *p*-Aminosalicylate with Several *p*-Aminosalicylic Acid Preparations

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*p*-Aminosalicylic acid (PAS) is administered, usually in conjunction with isoniazid or streptomycin, in the treatment of pulmonary or extrapulmonary tuberculosis to prevent or postpone the development of resistance to these chemotherapeutic agents by the tubercle bacillus. In addition, Morse and his co-workers<sup>1</sup> have demonstrated that PAS given with isoniazid may increase the blood levels of unconjugated isoniazid by consuming part of the acetylating capacity of the body. Brodersen<sup>2</sup> et al have shown that phenyl *p*-aminosalicylate (phenyl PAS) has an in vitro tuberculostatic effect comparable to dihydrostreptomycin and superior to PAS.

Although PAS is extremely valuable in the prolonged chemotherapy of tuberculosis, its usefulness is often limited because of gastrointestinal disturbances, such as nausea, vomiting, abdominal cramps, diarrhea, and anorexia. Various attempts to decrease the gastrointestinal irritation caused by PAS have been only partially successful. These efforts include giving divided doses after or during meals; using sodium, potassium, and calcium salts as well as the ascorbic conjugate of PAS; administration of buffered, enteric-coated, or granular forms of the acid; and the concomitant use of probenacid to increase absorption or delay excretion and permit the administration of smaller quantities of PAS.

Previous studies<sup>3-6</sup> have indicated that a *p*-aminosalicylate anionic exchange resin complex (Rezipas) was tolerated with less difficulty than other preparations tested and produced clinically effective serum levels.

The present preliminary report is concerned with the toleration and absorption of phenyl *p*-aminosalicylate in comparison with the anionic exchange resin complex of *p*-aminosalicylic acid and the sodium, calcium, and potassium salts of PAS.

## MATERIALS AND PROCEDURES

Phenyl *p*-aminosalicylate is the phenyl ester of PAS and was first described by Freire.<sup>7</sup> It is a white crystalline substance with the composition  $C_{13}H_{11}NO_3$  and a molecular weight of 229. One Gm. of phenyl *p*-aminosalicylate supplies the equivalent of 0.67 Gm. of PAS. It was administered orally in doses of 4 Gm. three times daily, before meals, each dose providing 2.68 Gm. of PAS. The anionic exchange resin complex of PAS was given orally in doses of 6 Gm. four times daily providing 3 Gm. of *p*-aminosalicylic acid. The sodium, calcium, and potassium salts of PAS were administered in doses of 3 Gm. four times daily, after or during meals, this regimen providing, respectively, 2.64, 2.16, and 2.01 Gm. of PAS per dose. In most patients, isoniazid, in daily doses of 4 to 5 mg./Kg. of body weight, was administered in conjunction with these preparations.

In the studies of toleration and absorption, 343 tuberculous patients were observed with each subgroup having received one of five PAS preparations. The patients were seen at least weekly for 2 to 12 months with the exception of the group

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TABLE I  
Number and Severity of Symptoms

	Anionic exchange resin complex of PAS		Calcium PAS		Sodium PAS		Potassium PAS		Phenyl PAS	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total number	117	100.0	81	100.0	71	100.0	49	100.0	25	100.0
No symptoms	67	57.3	28	34.6	27	38.1	8	16.3	22	88.0
Mild symptoms	40	34.2	32	39.5	26	36.6	15	30.6	2	8.0
Moderate symptoms	9	7.7	16	19.7	15	21.1	24	49.0	1	4.0
Severe symptoms	1	.8	5	6.2	3	4.2	2	4.1	0	0.0

receiving phenyl *p*-aminosalicylate who were observed weekly for two to five months at the time this report was prepared.

Serum levels of PAS were determined in fresh blood samples taken one to four hours after the initial morning dose. The blood samples were assayed by the Marshall modification of the Bratton-Marshall test.<sup>8</sup>

The clinical efficacy of phenyl *p*-aminosalicylate is not being reported at this time because the observation period with this drug is not sufficiently prolonged. The clinical results with *p*-aminosalicylate anionic exchange resin complex, and the sodium, calcium, and potassium salts of PAS have been reported previously.<sup>3</sup>

## RESULTS

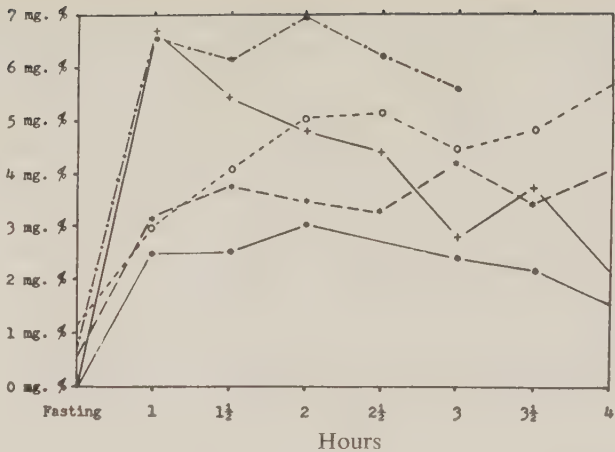
*Tolerance.* There were no gastrointestinal disturbances in 22 of 25 patients receiving phenyl *p*-aminosalicylate, mild symptoms were reported by 2 patients and moderate symptoms were present in 1 patient. None of the 25 patients had symptoms severe enough to require permanent discontinuation of the drug or decrease of the daily dosage.

Of 117 patients receiving *p*-aminosalicylate anionic exchange resin complex, gastrointestinal symptoms were absent in 67, mild in 40, moderate in 9, and severe in only 1 patient. Of 81 patients receiving calcium *p*-aminosalicylate, 28 reported no symptoms, 32 mild, 16 moderate, and 5 severe symptoms. Gastrointestinal symptoms were absent in 27 of the 71 patients receiving sodium *p*-aminosalicylate, mild irritation was noted in 26 patients, moderate symptoms in 15, and severe disturbances in 3 patients. Of 49 patients taking potassium *p*-aminosalicylate, gastrointestinal symptoms were absent in 8 patients, mild in 15, moderate in 24, and severe irritation in 2 patients. These results are summarized in table I.

*Absorption.* The average serum levels of PAS obtained with single doses of the various preparations are shown in figure 1. Following a 4 Gm. dose of phenyl *p*-aminosalicylate which supplied 2.68 Gm. of PAS equivalent, maximum serum levels of approximately 3 mg. of PAS per 100 ml. of serum were attained within two hours, and dropped slowly from the peak level for the duration of the observation period.

The maximum serum level obtained after a 6 Gm. dose of anionic exchange resin complex of *p*-aminosalicylic acid, which supplied 3 Gm. of PAS, was approximately 4 mg./100 ml. Peak levels were attained within approximately one to one and one half hours and remained essentially constant for the duration of the observation period. The maximum serum levels obtained after single 3 Gm. doses of sodium and potassium *p*-aminosalicylate were approximately 7 mg./100 ml. These peak levels

FIG. 1. The serum levels of acid PAS with various PAS salts are illustrated. A.M. after 4 Gm. for phenyl PAS; 3 Gm. for calcium, sodium, and potassium PAS; and 6 Gm. for anionic exchange resin of PAS. .— . potassium PAS; o - - - o calcium PAS; \* — \* anionic exchange resin of PAS; + — + sodium PAS; — phenyl PAS.



were reached within one hour and were maintained by the potassium preparation but decreased quite rapidly in the case of the sodium *p*-aminosalicylate. Peak levels of more than 5 mg./100 ml. were reached slowly with calcium *p*-aminosalicylate and remained relatively constant.

As shown in table II, the absorption of these preparations varied within rather wide limits from one patient to another and from day to day in the same patient, although the variation was less marked in the latter situation. A total of 873 determinations of serum blood levels for the various preparations have been completed.

### DISCUSSION

Phenyl *p*-aminosalicylate was well tolerated with only 3 of 25 patients having any gastrointestinal disturbances. Of these, symptoms were mild in 2 patients, moderate in 1 patient and none of the patients experienced severe symptoms. These observations are considered preliminary, since the number of patients is relatively few and the observation period is not sufficiently prolonged.

Previous studies<sup>3</sup> have indicated that there is no direct relationship between the dosage of PAS equivalent ingested and toleration. The potassium salt, in the dosage employed, supplied the least amount of PAS but caused gastrointestinal disturbances of some degree in 83.7 per cent of the patients. A single dose of the anionic ex-

TABLE II  
*Ranges of PAS Serum Levels in Mg. Per Cent\**

	Anionic exchange resin complex of PAS			Calcium PAS			Sodium PAS			Potassium PAS			Phenyl PAS		
	High	Low	Av.	High	Low	Av.	High	Low	Av.	High	Low	Av.	High	Low	Av.
Fasting	0.6	0.2	0.50	3.9	0.0	1.04	1.0	0.0	0.26	3.0	0.0	0.52	—	—	—
1 hour	5.8	1.0	3.10	8.2	1.4	4.02	10.8	4.0	6.78	12.0	1.8	6.76	4.8	1.2	2.48
1½ hours	8.4	0.8	3.73	9.2	1.0	4.07	9.3	3.0	5.44	11.2	1.2	6.20	3.2	1.8	2.50
2 hours	9.1	1.2	3.55	9.8	2.2	5.07	8.4	2.0	4.85	11.3	2.4	7.00	4.0	1.2	3.05
2½ hours	7.2	1.8	3.30	9.0	2.2	5.13	6.4	3.2	4.48	9.2	3.6	6.26	—	—	—
3 hours	8.4	1.4	4.22	7.6	1.6	4.46	6.0	0.8	2.76	8.8	2.2	5.60	4.0	1.0	2.37
3½ hours	5.6	1.0	3.45	4.8	4.8	4.80	5.2	1.0	3.80	—	—	—	2.2	2.2	2.20
4 hours	5.6	1.2	4.01	9.0	3.5	5.65	7.2	1.0	2.13	—	—	—	1.6	1.6	1.60
No. determinations	197			184			207			247			38		

\* A.M. after 6 Gm. for anionic exchange complex of PAS; 3 Gm. for calcium, sodium, and potassium; 4 Gm. for phenyl PAS.

change resin complex of *p*-aminosalicylic acid on the other hand, supplied the largest amount of PAS, but produced fewer symptoms. Calcium and potassium *p*-aminosalicylates were intermediate in this regard, producing symptoms in 65.4 and 61.9 per cent of patients, respectively.

There was no correlation between the rate and degree of absorption and the PAS equivalent content of each preparation. The rate and degree of absorption was apparently greatest with potassium *p*-aminosalicylate even though the potassium salt, in the dosage employed, contains the least amount of PAS equivalent of all the preparations tested. Phenyl *p*-aminosalicylate produces serum levels somewhat lower than those attained with the other preparations and further studies are in progress to determine the clinical significance of this finding. A greater number of tests will be necessary to obtain statistically significant data in this regard. The critical serum level required to produce the desired clinical effect is not known.

#### SUMMARY

1. Phenyl *p*-aminosalicylate was found to be well tolerated in a series of 25 patients with pulmonary tuberculosis. Only 3 patients in this group exhibited mild or moderate gastrointestinal disturbances.

2. The toleration of phenyl *p*-aminosalicylate was compared with the anionic exchange resin complex of *p*-aminosalicylic acid and sodium, potassium, and calcium *p*-aminosalicylate in a series of 343 tuberculous patients.

3. No relation could be demonstrated between toleration and the *p*-aminosalicylic acid equivalent per dose of the preparation tested, since potassium *p*-aminosalicylate which supplied the least amount of *p*-aminosalicylic acid equivalent produced the greatest number of gastrointestinal disturbances.

4. Studies on the rate and degree of absorption following comparable single doses indicated that no correlation could be demonstrated between absorption and the *p*-aminosalicylic acid content of the preparation, since the potassium salt, which was most rapidly and extensively absorbed, supplied the least amount of *p*-aminosalicylic acid equivalent per dose.

5. Further studies are in progress to determine the clinical effectiveness of phenyl *p*-aminosalicylate in relation to the serum blood levels attained with the dosage employed.

#### BIBLIOGRAPHY

1. MORSE, W. C.; CURRY, F. J.; MORSE, P. D.; CHAMBERS, J. S., AND LINCOLN, A. F.: Effect of oral PAS on biologically active isoniazid serum levels. Transactions 15th Conference on the Chemotherapy of Tuberculosis. Veterans Administration—Army and Navy, 1956, p. 283.
2. BRODERSEN, R.; BUNCH-CHRISTENSEN, K., AND TYBRING, L.: Tuberculostatic properties of *p*-aminosalol (phenyl *p*-aminosalicylate), *Acta pharmacol. et toxicol.* 11:307, 1955.
3. COHEN, S. S.; YUE, W. Y., AND TSAI, S. H.: Comparison of toleration, absorption and clinical effect of potassium PAS, sodium PAS, calcium PAS and Rezipas. Transactions 17th Conference on The Chemotherapy of Tuberculosis. Veterans Administration—Armed Forces, 1958, p. 129.
4. SMALL, M. J.; KIRMAN, D., AND ALLEN, R., JR.: Serum concentration studies with sustained-action para-aminosalicylic acid tablets, *Am. Rev. Tuberc.* 77:184, 1958.
5. SHANE, S. J., AND KRZYSKI, T. K.: The use of a para-aminosalicylic acid-resin combination in the treatment of tuberculosis, *Canad. M.A.J.* 72:137, 1955.
6. HOLLANDER, A. G.: Para-aminosalicylic acid-resin complex; studies in absorption, serum electrolytes, and tolerance, *Am. Rev. Tuberc.* 72:548, 1955.
7. FREIRE, S. A.: Activité tuberculostatique du para-aminosalicylate de phenyle (FR 7) sur le bacille tuberculeux in vitro et sur la tuberculose de la souris, *Compt. rend. Acad. d. sc.* 231:728, 1950.
8. MARSHALL, E. K.: Determination of para-aminosalicylic acid in blood, *Proc. Soc. Exper. Biol. & Med.* 68:471, 1948.

# "All-purpose" Penicillin in the Treatment of Gonorrhea: Increasing Failure Rates with Repository Penicillins

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Although syphilis shows every sign of being adequately controlled wherever penicillin has been widely used, the situation regarding gonorrhea is far less satisfactory. In many countries the immediate postwar decline has either now ceased or the numbers of cases are again rising. In the clinics of England and Wales, for example, Ministry of Health figures show that there has been an increase of 34.8 per cent in the numbers of new cases of gonorrhea in both sexes since 1951.

This failure to control gonorrhea is not confined to the United Kingdom. A similar experience has been obtained in other countries, including Australia,<sup>1</sup> Canada,<sup>2</sup> Denmark,<sup>3</sup> Finland,<sup>4</sup> France,<sup>5</sup> Hong Kong,<sup>6</sup> India,<sup>7</sup> Poland,<sup>8</sup> Norway,<sup>9</sup> and Sweden,<sup>10</sup> to name but a few.

## DECLINE IN EFFECTIVENESS OF REPOSITORY PENICILLINS

*Clinical.* This increase in the attack rate of gonorrhea has been accompanied by a deterioration in the effectiveness of repository penicillins, in spite of a world-wide tendency to increase the dosage of the antibiotic. Thus Willcox<sup>11</sup> reported only 4.6 per cent of failures in 226 male gonorrhea patients given 0.3 megaunits of procaine penicillin with aluminum monostearate (PAM) in 1952, but no less than 17.9 per cent in an equally large series in which double the amount was given in 1956. In 1957,<sup>12</sup> in a smaller series, a 17.9 per cent failure rate was noted with 1.2 megaunits. Similarly, with benzathine penicillin in 1957 a 27.4 per cent failure rate was obtained in 264 patients given single doses of 0.3 to 1.2 megaunits, whereas cure rates exceeding 95 per cent had earlier been reported in the United States.<sup>13</sup>

In Great Britain in recent years, there has been a large immigration of Negroes, particularly from the West Indies. The Negro population in Great Britain is currently estimated by the Home Office at 100,000. In an ethnic minority such as this, separated from their wives and away from home, venereal diseases are common, and a very large percentage of the gonorrhea cases encountered today are in this group. In 1952, in sample series studied by the author, less than 1 in 10 of gonorrhea cases were in Negroes. In 1957, more than one-half were in Negroes.

A striking feature is that the failure rates in Negroes are twice as high as those in other groups. Thus, in a series of 426 non-Negro patients treated in 1956–1957 with procaine penicillin, procaine penicillin with aluminum monostearate, or benzathine penicillin—all by injection—or with phenoxymethyl penicillin by mouth, or with streptomycin by injection, there were 12.0 per cent of failures. Of 454 Negroes similarly treated, the failure rate was 25.2 per cent.

It is considered probable that a significant proportion of the additional failures in the Negroes is due to a too early return to the same or similar sexual environment (i.e., reinfection), although the possibility cannot be overlooked that less sensitive strains of gonococci are being encouraged to develop more quickly in this group as a result of the frequent treatment with penicillin of the many repeated reinfections that occur.

The declining powers of repository penicillins in spite of increasing dosages has

been observed in other countries of the world, notably the Far East<sup>14</sup> including Hong Kong<sup>15</sup> and Japan,<sup>15</sup> but few reports are so far available from the United States.

*Pathological.* The gonococcus has a wide range of sensitivity. Love and Finland,<sup>16</sup> in a study of 286 strains, admittedly a few years ago, found that 95 per cent required only 0.03 unit of penicillin per ml. for inhibition, but that 3.5 per cent required 0.06 unit; 0.7 per cent, 0.13 unit; and 0.7 per cent, 0.33 unit. These workers, having undertaken such tests over a nine year period, found no increase in the proportion of resistant strains in the United States. Thayer et al.,<sup>17</sup> in an examination of 96 strains of gonococci, also in the United States, found a range of penicillin sensitivity of 0.005 to 0.2 unit ml., with a mean of 0.052 unit. Although the mean sensitivity was higher in patients who subsequently failed to respond to penicillin, these workers did not consider that they had found an explanation of penicillin failures and concluded that these must arise from a failure of the antibiotic to come into contact with the organisms.

In Great Britain, King<sup>18</sup> and Wilkinson and Curtis<sup>19</sup> showed the wide range of penicillin sensitivity of the gonococcus in patients prior to treatment in London. When the results of treatment were related to the penicillin sensitivity of the gonococcus before treatment, it was clear that the bulk of the failures occurred in those patients whose strains were the least sensitive before treatment, indicating that the treatment regimen produced a penicillemia that was inadequate to deal with them. As comparable regimens have been clinically more successful in the past, the conclusion must surely be, as far as Great Britain is concerned, that the number of less sensitive strains in circulation has in all probability increased, although there is no direct pathological evidence available to confirm or refute this.

#### ALTERNATIVE TREATMENTS

*Other Antibiotics.* If repository penicillins no longer give satisfactory results in the treatment of gonorrhea, the alternatives are to change to other antibiotics or to use quicker-acting penicillin preparations, which produce higher peaks of penicillemia sufficient to overcome the less sensitive strains of gonococci.

There are many antibiotics effective in gonorrhea, notably streptomycin, tetracycline (with and without oleandomycin), oxytetracycline, chlortetracycline, chloramphenicol, erythromycin, and spiramycin. Except for streptomycin, these are expensive and require 2.0 Gm. a case. Many persons believe that erythromycin should not be used for a relatively mild disease like gonorrhea but should be reserved for fulminating cases of staphylococcal septicemia. Moreover, if these other antibiotics were used on a wide scale, it is likely that they too in time would become less effective, for already with most of the orally administered antibiotics there is a failure rate in gonorrhea of about 10 per cent. Cases of gonorrhea resistant to the tetracycline antibiotics have been reported.<sup>20</sup> Streptomycin is the most promising alternative, since it is the cheapest and is capable of curing gonorrhea in a single injection. Indeed, it has been widely used in France for a number of years for gonorrhea in preference to penicillin. Cases of gonorrhea resistant to streptomycin have already been reported, however, by Davey,<sup>21</sup> and it is likely that the gonococcus might readily become less sensitive to streptomycin also.

*Quicker-acting Penicillins.* It is a widely held view, expressed for example at the World Health Organization Venereal Diseases Seminar held in Tokyo in March, 1958, that even if penicillin were only 70 per cent effective in gonorrhea, it would still be a good drug and that its routine use should be retained for the time being.

To make penicillin more effective in gonorrhea and to provide a higher peak of penicilemia capable of dealing with all of the less sensitive variants of gonococci, quicker-acting preparations are required. Ideally, perhaps, crystalline penicillin would provide the optimum curve by producing a quick rise and a quick fall and offering little opportunity for gonococci to be subjected to lower and surmountable levels of penicillin for long periods of time. To achieve this, however, multiple injections would be required at two to four hour intervals, and it is unlikely that patients could be persuaded to return to a regimen such as this, which was in use when penicillin was first introduced. The alternative, then, is procaine penicillin, given preferably in two large doses each of 1.2 megaunits at 12 to 24 hour intervals. Such a treatment would probably keep failure rates to a minimum and, if widely used, would tend to slow down any drug resistance to the antibiotic. With single injections of 0.6 megaunit of procaine penicillin, failure rates of 11.2 per cent have been obtained, 15.9 per cent in Negroes and 5.7 per cent in non-Negro patients.

#### EPIDEMIOLOGICAL CONSIDERATIONS OF TREATMENT

The general abandonment of repository penicillins, however, would entail the loss of two epidemiological advantages. The prolonged "penicillin tail" obtained with their use has been found of value in preventing reinfection while the consorts are being secured for examination and treatment. Without such a "tail," there is the fear that the higher cure rates obtained with quicker-acting preparations would be offset by a substantial increase in early reinfections. Also, the penicillin "tail" has been considered of possible value in reducing the reservoir of syphilis,<sup>22</sup> and large doses (1.2 megaunits of PAM) have been deliberately used in the treatment of gonorrhea in British Columbia with this objective in mind.<sup>23</sup>

#### USE OF MIXED PENICILLIN PREPARATIONS

The deliberate use of the penicillin "tail" was adopted by Hookings and Graves<sup>24</sup> in Memphis, Tennessee. Although 600,000 units of PAM was proving satisfactory in gonorrhea of the male, the number of cases being encountered was not declining, and "it became apparent that some form of therapy would have to be introduced which would render the male incapable of being reinfected until the disease in his female contacts was cured." For this reason 1.2 megaunits of benzathine penicillin was added to the 0.6 megaunit of PAM, at first in women (and later also in men), and subsequently there was a striking fall in the number of gonorrhea cases seen in the district.

The same principle was endorsed by Schamberg et al<sup>25</sup> in Philadelphia, who, in an effort to reduce the gonorrhea attack rate, gave approximately 2400 men patients and also 1700 female contacts 2.4 megaunits of benzathine penicillin. The women were asked to return for identical treatment every eight weeks. Although the failure rates were assessed at only 0.5 per cent of the women and 0.25 per cent of men, and such a dose was calculated to provide a penicilemia for 50 days sufficient to kill 95 per cent of strains of gonococci, it was considered insufficient to kill the most resistant strains. In order to prevent dissemination of these more resistant organisms, a preparation providing a higher initial blood level was considered advisable and a mixture of 600,000 units of procaine penicillin and 1.2 million units of benzathine penicillin is currently being used in the venereal diseases control program.

A number of triple penicillin combinations are on the market, commonly containing 300,000 units of crystalline benzyl penicillin, 300,000 units of procaine penicillin, and 600,000 units of benzathine penicillin. With 1.2 megaunits of such a preparation, cure rates of 97 per cent were reported by Sleath and Nelson<sup>26</sup> in Canada, although similar results were encountered with 1.2 megaunits of PAM. Wherrett et al,<sup>27</sup> in the United States, also had 98.2 per cent success in a large series treated.

#### A BRITISH STUDY OF ALL-PURPOSE PENICILLIN

Both series just mentioned refer to the North American continent, where, as has already been noted, the problem of penicillin failures in gonorrhea, judging by the literature, does not seem so acute as in Britain. It was considered desirable therefore to use an "all-purpose" preparation under British conditions. The preparation used was such that each ampoule contained 300,000 units of crystalline potassium penicillin, 300,000 units of procaine penicillin G, and 600,000 units of benzathine penicillin,\* being dissolved in 2 ml. of distilled water and the whole being given by intramuscular injection.

#### CASE MATERIAL

Ninety-five men with acute, uncomplicated gonorrhea have been treated with single injections of 1.2 megaunits of the all-purpose penicillin combination. Fifty-five patients were Negroes (2 from West Africa, 1 from the Sudan, 1 from Somaliland, 1 from the United States, and the remainder from the West Indies). Forty were non-Negro patients, of whom 21 were from the United Kingdom, 8 from Ireland, and 1 each from Australia, Canada, Greece, Hungary, Lithuania, Malta, Pakistan, Poland, and Romania, and 2 from India.

The average age was 27.2 years (with a range from 17 to 50 years) being 26.8 for the Negroes (range from 19 to 39) and 27.3 years for the non-Negro patients (range from 17 to 50). Eighty-one of the patients were single and 14 were married. Of the Negro patients, 16.4 per cent were married (although the majority were not living with their wives), and of the non-Negro patients, 12.5 per cent were married.

Forty-three patients had had no previous venereal disease, but the remaining 52 had had no less than 124 previous attacks of gonorrhea, 20 of nongonococcal urethritis, 4 of syphilis, 2 of balanitis, and 1 of scabies. Of the Negro patients, 25 (45.5 per cent) had had no previous venereal incident, while the 55 patients in the group had had 95 previous incidents (average of 1.7 each). Eighteen of the non-Negroes (45.0 per cent) had had no previous incident, and the 40 patients in the group had had a total of 56 previous incidents (average of 1.4 each).

The discharge had been present before treatment for one to three days in 69, for four to seven days in 24, and for more than seven days in 2. The Negro patients as a whole reported somewhat less promptly to hospital, for 36 of the 55 patients (65.5 per cent) had had the discharge for three days or less compared with 33 (82.5 per cent) of the 40 non-Negro patients. All but 20 of the patients complained of some urinary discomfort, all but 9 of the non-Negro patients (22.5 per cent) and all but 11 of the Negro patients (20.0 per cent).

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\* The trade name of Wyeth Laboratories for a combination of crystalline potassium penicillin, procaine penicillin G, and benzathine penicillin is Penidural-All-Purpose.

The infection had been acquired from a stranger (including prostitutes) in 60, from a friend in 32, and from the wife in 3. A stranger was responsible in 55 per cent of non-Negro patients and in 69.1 per cent of Negro patients. The incubation period was apparently one to three days in 47, four to seven days in 24, 8 to 14 days in 16, and more than 14 days in 8. In 30 of the Negroes (54.5 per cent) the apparent incubation period was one to three days as compared with

TABLE I  
*Comparison of Basic Data of Two Groups*

	Negroes (55)	Non-Negroes (40)	Total (95)
Age, yr.			
Average	26.8	27.3	27.2
Extremes	19-39	17-50	17-50
Marital Status			
Single	46	35	81
Married	9	5	14
% married	16.4	12.5	14.7
Previous Venereal Disease			
None	25	18	43
% with none	45.5	45.0	45.3
Gonorrhea	80	44	124
Nongonococcal urethritis	12	8	20
Syphilis	2	2	4
Scabies	1	—	1
Balanitis	0	2	2
Total attacks	95	56	151
Average no. attacks	1.7	1.4	1.6
Duration of Discharge, days			
1-3	36	33	69
4-7	17	7	24
More than 7	2	—	2
% 1-3	65.5	82.5	72.6
Presence of Dysuria			
Yes	44	31	75
No	11	9	20
% no	20.0	22.5	21.1
Source of Infection			
Stranger	38	22	60
Friend	15	17	32
Wife	2	1	3
% stranger	69.1	55.0	63.2
Apparent Incubation Period, days			
1-3	30	17	47
4-7	12	12	24
8-14	7	9	16
More than 14	6	2	8
% 1-3	54.5	42.5	49.5
% more than 14	10.9	5.0	8.4
Serum Tests for Syphilis			
Wassermann      VDRL or Kahn			
Negative      Negative	40	37	77
Negative      Positive	12	2	14
Positive      Negative	—	1	1
Positive      Positive	3	—	3
% negative	72.7	92.5	81.1
Gonococcal Complement Fixation Test Results			
Not done	8	2	10
Positive	6	—	6
Weakly positive	9	3	12
Anticomplementary	2	—	2
Negative	30	35	65
% negative	63.8	92.1	76.5

TABLE II  
*Follow-up and Results*

Follow-up	Followed	Relapse	Reinfection	Nongonococcal infection
0	95	—	—	—
1-3 days	87	—	—	1
4-7 days	80	4	—	—
8-14 days	64	2	—	1
15-21 days	56	—	1	2
22-28 days	47	—	1	3
1-2 mo.	40	—	3	3
2-3 mo.	18	1	5	1
More than 3 mo.	8	—	6	—
Total followed	87	7	16	11

17 (42.5 per cent) of the non-Negroes. Exceptionally long incubation periods (more than 14 days) were reported by 6 Negroes (10.9 per cent), compared with 2 (5.0 per cent) non-Negroes.

Gonococci were recovered by smear in all cases prior to treatment. The Wassermann and Kahn (or Venereal Diseases Research Laboratory) tests were both negative in 77 cases, both positive in 3; the Wassermann reaction was negative but the other test gave a positive result in 14, and in 1 case the Wassermann test was positive and the Kahn test was negative. Complete seronegativity in regard to syphilis was obtained in 37 of the non-Negro patients (92.5 per cent) compared with only 40 (72.7 per cent) of the Negro patients—doubtless due in many cases to past yaws infections in the latter group. The gonococcal complement-fixation test was performed in 85 cases, the result being strongly positive in 6, weakly positive in 12, anticomplementary in 2, and negative in 65. Of the 38 non-Negroes on whom the test was done, a negative result was obtained in 92.1 per cent as compared with 63.8 per cent of the Negroes, or 30 negative results in 47 persons tested.

The material data in the two groups are compared in more detail in table I. In general, the age distribution and marital status were much the same, as was the incidence of dysuria. The Negroes had had more previous venereal disease, and their discharges had been present on the average somewhat longer before treatment. The source of infection was a stranger more frequently in the Negro group, in which was found a higher incidence of non-negative serum reactions for syphilis (doubtless in many instances due to past yaws) and a higher incidence of non-negative gonococcal complement-fixation reactions.

#### FOLLOW-UP AND RESULTS OF TREATMENT

All patients were given a single injection of 1.2 megaunits and were instructed to return within two to four days and thereafter at approximately one week, and then two weeks later when a prostatic massage was made and the secretion examined. Those attending after this time were seen approximately monthly for a further two months, a final serum test for syphilis being made at the end of three months before the patient was discharged. By no means all patients attended for the time prescribed, but sufficient time to allow them to do so had elapsed before an assessment of the results was made. The follow-up and results obtained in the whole series are given in table II.

TABLE III  
*Results in Negroes and Non-Negroes*

Race	Treated	Followed	Relapse	Reinfection	Nongonococcal infection	% relapse of those followed
Negro	55	51	5	12	7	9.8
Non-Negro	40	36	2	4	4	5.6
Total	95	87	7	16	11	8.0

Of 95 patients treated, 87 were followed-up and a relapse was reported in 7 (8.0 per cent). Six of the relapses occurred within 14 days and one at 77 days. Although further sexual exposure was denied in the latter case, reinfection was strongly suspected. All 16 reinfections occurred late: 6 after the stipulated observation period of three months and 14 after one month. Further sexual exposure was admitted by all, but in only a few was it possible to examine the consort. Nongonococcal infections were noted in 11 (12.6 per cent).

As in other series reported,<sup>11,12</sup> the failure rates were higher in Negro than in non-Negro persons (table III).

The results in both groups were certainly a considerable improvement on those previously quoted as having been obtained with repository penicillins alone and were somewhat better than those obtained with single injections of 0.6 megaunit of procaine penicillin, which had showed 15.9 per cent of failures in Negroes and 5.7 per cent in non-Negroes (over-all 11.2 per cent).

Apart from the improved results, the use of an all-purpose preparation has the epidemiological advantages previously discussed of the "penicillin tail." On the other hand, it must be admitted that the presence of a prolonged low-level of penicilemia might itself encourage further selective breeding of the less sensitive strains of gonococci if the patients were re-exposed to gonorrhea during the time that it is present. Also, the fact that 5.6 to 9.8 per cent of failures still occur with its use indicates that further lessened sensitivity of the gonococcus to penicillin is likely to occur, although at a slower rate.

#### SUMMARY AND CONCLUSIONS

1. The failure to control gonorrhea throughout the world is noted. At the same time as there has been an increase in the attack rate in many areas, there have been some signs of increasing failure rates with repository penicillins in spite of a general increase in dosage.

2. In such experience reported from London, it is considered likely that the dissemination of strains of gonococci less sensitive to penicillin accounts for many of the failures, although the situation is confused by higher failure rates being noted in an ethnic minority, which might also be partly accounted for by the same reason.

3. Possible measures to meet the situation are considered. As repository penicillins are no longer sufficiently effective, it is considered necessary, if penicillin continues to be used, to administer a quicker-acting preparation providing a sufficiently high peak of penicilemia to overcome the less sensitive strains.

4. The repository penicillins have been considered to have an epidemiological advantage, since the longer penicilemia (sufficient to overcome the majority if not all strains of gonococci) assists in preventing reinfection while the consorts

are secured and also in reducing the reservoir of syphilis. Mixed preparations, containing both quick-acting and slow-acting penicillins, may therefore have a place in this field, although there is still the objection that further lessened sensitivity to penicillin might be fostered by selection of less sensitive strains if patients re-expose themselves to gonorrhea while the low levels of penicillin are still being carried.

5. A report is made of 95 patients treated with 1.2 megaunits of an all-purpose preparation with 300,000 units of crystalline potassium penicillin, 300,000 units of procaine penicillin G, and 600,000 units of benzathine penicillin. Of the 95 patients treated, 87 were followed. There were 7 relapses (8.0 per cent), 6 of which occurred within two weeks, and 16 reinfections after longer periods of time up to several months. Eleven patients (12.6 per cent) were treated for nongonococcal urethritis during the period of observation. Of the Negro patients followed, treatment failed in 9.8 per cent, compared with 5.6 per cent of the non-Negro group. These immediate results were better than those obtained with other methods in recent routine use.

6. Even if 1.2 megaunits of "all-purpose" penicillin does give results that are an improvement on other methods, some failures do occur with its use. As long as some failures continue to occur, it is considered likely that the present situation of deteriorating results and an increased attack rate will continue. The process can, however, be slowed down if improved results are obtained.

#### BIBLIOGRAPHY

1. JOHNSON, H. C.: Paper presented at World Health Organization Venereal Diseases Seminar, WHO Working Document WPR/VD/3, Tokyo, 1958.
2. LOSSING, E. H., AND ALLEN, A. H.: Venereal disease in Canada, *Brit. J. Ven. Dis.* 32:150-153, 1956.
3. ELIOT, T. D.: Norway conquers venereal disease, *Brit. J. Ven. Dis.* 31:2-8, 1955.
4. HÄRÖ, A. S., AND PÄTÄLÄ, R.: Epidemiology of gonorrhoea in Finland 1953 to 1955, *Brit. J. Ven. Dis.* 33:70-77, 1957.
5. DUREL, P.: Survey of venereal disease situation in France, *Brit. J. Ven. Dis.* 32:145-149, 1956.
6. THOMPSON, G. M.: Paper presented at World Health Organization Venereal Diseases Seminar, WHO Working Document WPR/VD/4, Tokyo, 1958.
7. RAJAM, R. V.: Venereal diseases in India, *Brit. J. Ven. Dis.* 32:79-81, 1956.
8. TOWPIK, J.: Problems of venereal disease control in Poland, *Brit. J. Ven. Dis.* 33:78-79, 1957.
9. GJESSING, H. C.: Venereal diseases past and present in Norway with special reference to Oslo, *Brit. J. Ven. Dis.* 32:86-90, 1956.
10. TOTTIE, M.: Venereal disease problems in Sweden today, *Brit. J. Ven. Dis.* 32:157-158, 1956.
11. WILLCOX, R. R.: Papers presented at World Health Organization Venereal Diseases Seminar, WHO Working Documents WPR/VD/39, 40, 41, Tokyo, 1958.
12. WILLCOX, R. R.: Treatment of gonorrhoea with streptomycin in London in 1956, *Acta dermat.-venereol.* 37:332-337, 1957.
13. O'BRIEN, V. F., AND SMITH, C. A.: *Am. J. Syph., Gonorr. & Ven. Dis.* 36:519-524, 1952.
14. ANON.: Venereal diseases symposia, *Lancet* 1:899, April 26, 1958.
15. WILLCOX, R. R.: "These dying diseases," *Lancet* 1:1127, May 24, 1958.
16. LOVE, B. D., JR., AND FINLAND, M.: Susceptibility of *Neisseria gonorrhoeae* to eleven antibiotics and sulfadiazine, *A.M.A. Arch. Int. Med.* 95:66, 1955.
17. THAYER, J. D.; FIELD, F. W.; MAGNUSON, H. J., AND GARSON, W.: The sensitivity of gonococci to penicillin and its relationship to "penicillin failures," *Antib. & Chemo.* 7:306, 1957.
18. KING, A. J.: "These dying diseases." Venereology in decline?, *Lancet* 1:651, March 29, 1958.
19. WILKINSON, A. E., AND CURTIS, E. R.: Paper presented before the Medical Society for the Study of Venereal Diseases, London, 1958.
20. STUHLERT, B.: Treatment of penicillin-resistant gonorrhoea, *Z. Haut. v. gesch.-Kr.* 22:189, 1957.
21. DAVEY, A. C. C.: Streptomycin-resistant gonorrhoea in the female, *Brit. J. Ven. Dis.* 33:179-180, 1957.

22. GUTHE, T.: Paper presented at World Health Organization Venereal Diseases Seminar, WHO Working Document WPR/VDT/43, Tokyo, 1958.
23. KANEE, B., AND NELSON, A. J.: Challenging trends in VD control, *Am. J. Syph., Gonorr. & Ven. Dis.* 38:354-360, 1954.
24. HOOKINGS, C. E., AND GRAVES, L. M.: Benzathine penicillin G in the control of gonorrhoea, *Brit. J. Ven. Dis.* 33:40-42, 1957.
25. SCHAMBERG, I. L.; KALODNER, A., AND LENTZ, J. W.: Antibiotic quarantine of gonorrhoea. I. Effect in females, *Brit. J. Ven. Dis.* 34:24-30, 1958.
26. SLEATH, G. W., AND NELSON, A. J.: Clinical evaluation of a new triple penicillin salt in the treatment of acute gonorrhoea, *Canad. J. Pub. Health* 47:383, 1956.
27. WHERRITT, H. R.; ALTHEIDE, H. E., AND DUFFETT, N. D.: Gonorrhea; treatment with a triple penicillin combination, *Missouri Med.* 53:767-768, 1956.

# Observations on the Prophylaxis of Ophthalmia Neonatorum in a Municipal Hospital

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The city of New York, as well as a number of other large municipalities in the United States, has recently relaxed the public health laws governing ophthalmic prophylaxis for the newborn infant. Among the factors contributing to these statutory revisions are the effectiveness of the broad-spectrum antibiotics in the treatment of venereal diseases and the increased utilization of trained medical personnel and hygienic controls implicit in the rise in prenatal care and hospitalization for delivery.

In the last decade various investigators have undertaken the re-evaluation of silver nitrate prophylaxis of ophthalmia neonatorum, which Crede introduced in 1880, and its comparison with newer techniques. Recommendations for the protection of the eyes of the newborn infant have ranged from abandonment of all medication to the application of new ophthalmic preparations and the systemic administration of antibiotics.

The general practice of the Crede procedure is historically credited with reducing the incidence of ocular infections. However, the introduction of silver nitrate solutions into the conjunctival sac has never been without disadvantages and some element of danger. The solutions employed may be unstable or contain free nitric acid. Severe injuries to ophthalmic tissues from the accidental use of solutions stronger than 1 or 2 per cent are a matter of record.

Even when the silver nitrate concentration is within safe limits, irritation of the tearless eyes of the newborn infant may produce conjunctivitis. Attempts to reduce this possibility by washing the eyes after the silver nitrate instillation eliminate the antiseptic value of the medication as well as its irritating effect.

Reports on the incidence of chemical conjunctivitis vary from as low as 4 per cent to 100 per cent, observed by Allen and Barrere<sup>1</sup> in a study of 653 infants who received a silver nitrate instillation at birth and again after a three hour interval. Kozinn and his associates,<sup>2</sup> in a review of the records of more than 1000 infants, found purulent conjunctivitis in 18 per cent.

Elliott<sup>3</sup> analyzed the records of more than a hundred institutions that dispensed with all medication, using only sterile water, normal saline, or dry swabbing for cleansing the eyes of the infant. The results were similar to or better than those with silver nitrate prophylaxis, with only two hospitals reporting an increased incidence of ophthalmia. Elliott concluded that bacterial contamination from the birth canal was not contributory, but that ophthalmia was due either to cross infection from the staff or nursery occupants or to mechanical irritation from such sources as lint from blankets.

Ophthalmic solutions, drops, and ointments in which penicillin, oxytetracycline, chlortetracycline, or bacitracin represented the antibacterial component have been tested by various investigators. A wide divergence is reflected in their judgments,

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TABLE I

*Silver Nitrate Ophthalmic Prophylaxis in the Newborn Infant in 1952*

Ophthalmic medication	Systemic medication	Number of cases	Incidence of chemical conjunctivitis	Incidence of nonspecific conjunctivitis	Incidence of gonorrheal ophthalmia
Silver nitrate, 1% solution	None	3165	15 (0.46%)	14 (0.44%)	0

some<sup>2,4,5</sup> finding the newer prophylactic preparations superior to silver nitrate, while others<sup>6,7</sup> concluded that simple washing of the eyes provided adequate protection.

For many years prior to 1957, a 1 per cent solution of silver nitrate, furnished in individual ampoules, was employed at Harlem Hospital. The solution was placed in each eye after they had been wiped with a sterile dry sponge. The eyes were then rinsed with sterile distilled water and wiped dry.

When the mandatory provision for the silver nitrate procedure was rescinded by the Department of Health, it was deemed advisable to evaluate the necessity for prophylactic measures and, if such protection was considered imperative, to determine the type most effective for our hospital population and conditions.

For purposes of comparison with a period in which the Crede method was the sole form of prophylaxis, the year 1952 was selected at random. In 3165 live births, 14 cases of nonspecific conjunctivitis and 15 cases of chemical conjunctivitis were recorded, and there was no instance of gonorrheal ophthalmia (table I).

Various approaches were involved in a four month study undertaken in 1957, the results of which have been previously reported.\* We employed for local medication an ophthalmic ointment\* prepared in 3.5 Gm. tubes for individual use, each containing 500 units of bacitracin per Gm. and 2 per cent phenacaine hydrochloride. The *Neisseria gonorrhoeae* is highly susceptible to this concentration of the antibiotic. This preparation has the additional advantage of stability at room temperature, permitting storage in the delivery room, and it is easily instilled into the eyes of the newborn infant.

Of 1144 live births in this period, penicillin was administered intramuscularly to 568 infants. This group was subdivided roughly into thirds, the first receiving no topical treatment, while the remaining two thirds had instillations of either bacitracin ointment or silver nitrate. Although the results were eminently satisfactory, there being no incidence of either nonspecific conjunctivitis or gonorrheal ophthalmia, we were not convinced of the desirability of routine systemic penicillin therapy. Inherent in this practice are dangers of sensitization of an entire age group in the community and the promotion of bacterial strains resistant to the antibiotic.

The remainder of the infants born during that study, 576, received no systemic medication. Bacitracin ointment was used for 277, while the others had no topical prophylaxis. There was no gonorrheal ophthalmia. Two cases of conjunctivitis occurred in the untreated group. We therefore concluded in this limited study that bacitracin ointment without systemic penicillin afforded adequate protection from ophthalmia neonatorum with no untoward reactions. However, since the number of cases was not of statistical significance, it was decided to continue the study.

From July 1, 1957, to June 30, 1958, there were 3355 live births. The infants

\* The trade name of The Upjohn Co. for this ointment is Baciguent ophthalmic ointment. The ointment was made available for this study by The Upjohn Co.

born on the numerically even days of the calendar received no medication for ophthalmia neonatorum, the eyes being swabbed with distilled water and wiped dry. For those delivered on the odd days, in addition to the mechanical cleansing of the eyes, we used the bacitracin-phenacaine ointment. In table II, the results of the previous four month trial are combined with data from this year-long investigation.

When only mechanical cleansing of the eyes was employed in 299 infants, as previously reported, 2 developed conjunctivitis of a nonspecific nature. In the extended study 1636 were similarly treated. There were 7 cases of nonspecific conjunctivitis and 5 cases of gonorrheal ophthalmia. Thus a total of 1935 (table II) received no topical medication, with an incidence of 9 cases of conjunctivitis and 5 of gonorrheal infection.

When, in the recent series of 1719 newborn infants, bacitracin ointment prophylaxis was added to the cleansing procedure, we found 4 cases of nonspecific conjunctivitis and 4 of ophthalmia neonatorum. These cases, added to the 277 similarly treated in the initial study, result in a total of 1996 infants, among whom there were 4 cases each of conjunctivitis and gonorrheal ophthalmic infection.

The total number of infants involved in both the preliminary and extended investigations was 3931. Conjunctivitis due to chemical irritation presented no problem. The nonspecific conjunctivitis observed in 13 infants was not severe and was of short duration. The 9 infants who had ophthalmia of gonorrheal origin were treated with intramuscular penicillin and continued local application of bacitracin ointment. All made an uneventful recovery with no corneal damage.

A comparison of table I, which shows no gonorrheal ophthalmia among 3165 newborn infants receiving silver nitrate prophylaxis, with table II, in which are recorded 9 such infections in 3931 cases, indicates that neither mechanical cleansing nor the use of bacitracin ophthalmic ointment provides the answer to the danger of ophthalmia neonatorum.

In the absence of legal regulations favoring a specific method of prophylaxis, the decision as to the procedure and medication logically devolves upon the department of obstetrics and should be based on a thorough knowledge of the health and hygiene status of the hospital community. Although the individual patient with gonorrhea coming under treatment at this hospital responds promptly to antibiotic therapy, the incidence of reinfection is high, and this disease has not been eradicated as a public health problem. We do not have the temerity to withhold from infants born here some form of protection against ophthalmia neonatorum.

TABLE II  
*Ophthalmic Prophylaxis in the Newborn Infant—1957 and 1958*

Ophthalmic medication	Systemic medication	Number of cases		Incidence of nonspecific conjunctivitis	Incidence of gonorrheal ophthalmia
None	None	Previously reported	299	2	0
		Present series	1636	7	5
		Total	1935	9 (0.46%)	5 (0.26%)
Bacitracin-phenacaine ointment	None	Previously reported	277	0	0
		Present series	1719	4	4
		Total	1996	4 (0.20%)	4 (0.20%)
		Total under study	3931		

We hold that the routine intramuscular administration of penicillin, although effective, involves the serious hazards of mass sensitization and the emergence of antibiotic-resistant variants.

It is our considered opinion therefore that some type of local prophylactic medication is necessary. Despite the chemical irritation of the eyes of the newborn infant that silver nitrate occasionally produces, an analysis of the results of the preliminary and present investigation leads to the conclusion that for our hospital situation a return to the Crede method of ophthalmic prophylaxis is justified.

#### SUMMARY

The advances represented by improved personal hygiene, the greater use of trained medical personnel, and the effectiveness of antibiotics in the control of venereal diseases have prompted a re-evaluation of the traditional procedures for prophylaxis of ophthalmia neonatorum. Recommended modalities vary from simple cleansing of the eyes to the application of topical antibiotic preparations and the routine use of intramuscular penicillin.

A study was undertaken in which the 1952 records of 3165 infants, protected by silver nitrate instillation, were compared with the results obtained during the years 1957 and 1958 in 1935 cases in which the eyes of the newborn infant were cleansed with sterile water and in 1996 infants for whom a bacitracin-phenacaine ointment was employed.

No case of gonorrheal ophthalmia was disclosed in the records of those infants treated with the traditional Crede method. There were 5 gonorrheal infections among those who had only mechanical cleansing of the eyes and 4 among those who received bacitracin ointment.

#### CONCLUSION

It appears that silver nitrate instillation, despite its considerable incidence of chemical conjunctivitis, remains the most effective and safe method of prophylaxis of ophthalmia neonatorum thus far employed at this hospital.

#### BIBLIOGRAPHY

1. ALLEN, J. H., AND BARRERE, L. E.: Prophylaxis of gonorrhea ophthalmia of the newborn, *J.A.M.A.* 141:522, 1949.
2. KOZINN, P. J.; MINSKY, A., AND SOLOMONS, E.: Oxytetracycline ophthalmic solution in the prophylaxis of ophthalmia neonatorum. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, pp. 307-312.
3. ELLIOTT, R. A.: Ophthalmia neonatorum. Review of prophylactic methods at present in use. *Month. Bull. Min. Health & Emerg. Pub. Health Lab. Serv.* 11:135, 1952.
4. DAVIDSON, H. H.: Penicillin in prophylaxis of ophthalmia neonatorum, *Obst. & Gynec. Surv.* 7:147, 1952.
5. CULLER, A. M., AND CLARK, S. G.: Aureomycin prophylaxis against ophthalmia neonatorum: second series, *Am. J. Ophth.* 34:982, 1952.
6. O'BRIEN, D.: Terramycin in the prophylaxis of ophthalmia neonatorum, *Lancet* 1:347, 1952.
7. LEFF, M.: Care of eyes in newborn. Report on 4500 cases, *West. J. Surg.* 59:170, 1951.
8. ANDERSON, G. D., AND POSNER, A. C.: Bacitracin ointment in the prophylaxis of ophthalmia neonatorum: a preliminary report. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 552-555.

# Benzathine Penicillin G in Oil

## A Clinical Evaluation in the Treatment of Early Syphilis

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Benzathine penicillin G was first introduced in 1951 as a repository preparation, which, in a dosage of 2,400,000 units, maintains a detectable blood level in most patients for a month or longer. A clinical evaluation conducted by the Public Health Service showed that a single injection of 2,400,000 units of benzathine penicillin G was equally as effective in the treatment of early syphilis as 4,800,000 units of procaine penicillin G in oil with 2 per cent aluminum monostearate (PAM).<sup>1</sup> The efficacy of this smaller dosage made benzathine penicillin G the first practical preparation for single-injection therapy for syphilis.

Today, benzathine penicillin G is one of the most widely used antibiotics in the field of venereal disease and is probably the drug of choice for the treatment of syphilis in the United States. For the domestic market, benzathine penicillin G is presuspended in aqueous solution, and if it is refrigerated, the expiration time allowed is two years. The product for export, however, requires reconstitution with a diluent and for this reason has proved impractical for fast field administration in eradication campaigns conducted by the World Health Organization.

To overcome this technical drawback, benzathine penicillin G in oil with 2 per cent aluminum monostearate (BOM) was developed. Storage stability data on this preparation indicate no loss of potency at room temperature (25 C.) or at body temperature (37.5 C) over a period of 4½ years. Although some discoloration takes place, at neither temperature is it more than that of "old ivory." This product has the added advantage of not caking in the vial, even though some separation of the suspension from the oil vehicle may occur.

In 1956 the Public Health Service was requested to conduct a clinical evaluation of this new preparation in terms of its effectiveness in the treatment of early syphilis. Interest was centered in a low dosage—one that would not be too extravagant for yaws eradication campaigns, where 600,000 units of PAM is the usual dosage, but one that would be sufficient to cure most syphilis in areas where the incidence of both diseases is high. In this country a minimum of 2,400,000 units of benzathine penicillin G or 4,800,000 units of PAM is recommended for the treatment of secondary syphilis.

On the basis of penicillin blood level assays performed by the Food and Drug Administration, a dosage of 1,200,000 units was selected for evaluation. On this dosage, all of 20 subjects maintained detectable blood concentrations for more than a week, the majority for more than two weeks. It should be added, for the benefit of those who are not too familiar with the treatment of syphilis, that it is the duration of penicillin concentrations rather than their height that largely determines the effectiveness of a preparation.

The clinical evaluation of 1,200,000 units of benzathine penicillin G in oil was

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Benzathine penicillin G in oil with 2 per cent aluminum monostearate (BOM) used in this evaluation was furnished by Wyeth Laboratories.

TABLE I  
*Factors Influencing Rate of Reaction to BOM*

	Total cases	Cases reacting	
		Number	Rate/1000
Total	868	18	20.7
Age			
Unknown	15	—	0.0
10-19	154	2	13.0
20-29	493	12	24.3
30-39	161	3	18.6
40-49	39	1	25.6
50 and older	6	—	0.0
Race and sex			
White male	60	4	66.7
White female	10	—	0.0
Nonwhite male	642	12	18.7
Nonwhite female	156	2	12.8
Previously reacted to penicillin	19	4	210.5
Did not react to penicillin previously	590	8	13.6
No previous penicillin	173	4	23.1
No data	86	2	23.3
Diagnosis			
Epidemiological treatment	91	1	11.0
Gonorrhea	670	13	19.4
Syphilis	107	4	37.4
Type of reaction			
Urticaria		12	13.8
Serum sickness		2	2.3
Anaphylactoid		1	1.2
Erythema multiforme		1	1.2
Generalized pruritus		1	1.2
Fever and joint pains		1	1.2

conducted through the cooperation of the health departments of Chicago, New York City, Detroit, St. Louis, Memphis, Atlanta, and Guilford County, N.C. One thousand injections of 1,200,000 units each were furnished the participating treatment facilities. It was hoped that during a three-month period 800 patients with gonorrhea and 200 patients with early syphilis could be treated. In order to observe reactions to treatment, it was requested that all patients treated be observed for a month and that patients treated for early syphilis be followed for at least one year. Due to program changes during the treatment phase of the study, only 868 of the 1000 injections distributed were utilized for evaluation purposes.

#### REACTIONS TO TREATMENT

Reactions to treatment were observed in 18 of the 868 patients, or 20.7/1000 treated. The type and frequency of reactions are shown in table I.

The reaction classified as "anaphylactoid" by the examining physician was described as follows: The patient was treated with 1,200,000 units of benzathine penicillin G in oil for gonorrhea. Within five minutes the patient complained of pruritus and burning sensation and was unable to stand. Dyspnea and vomiting accompanied by abdominal cramps then ensued. The patient was cold and clammy and, although not unconscious, appeared to be in shock. One-half ml. 1:1000 aqueous epinephrine subcutaneous and 10 mg. intravenous diphenhydramine were given with good response. The patient recovered in the next hour and a half and had no further difficulties.

Only 70 of the 868 patients treated were Caucasian and 4 of the reactions re-

TABLE II  
*Comparison of Incidence of Reactions to BOM and PAM*

Duration of observation, days	Total cases observed	% of non-reacting cases observed	Adjusted total cases observed	Patients experiencing reaction		
				Number	Rate/1000	Cumulative rate/1000
<i>BOM</i>						
1	868		868	2	2.3	2.3
2-4	707	81.64	709	4	5.6	7.9
5-7	681	96.87	687	1	1.5	9.4
8-14	624	91.76	630	11	17.5	26.9
15-21	492	80.26	506			26.9
22-28	448	91.06	461			26.9
More than 28	387	86.38	398			26.9
<i>PAM</i>						
1	12,179		12,179	15	1.2	1.2
2-4	3541	29.11	3545	14	3.9	5.1
5-7	3053	86.56	3069	8	2.6	7.7
8-14	2653	87.13	2674	43	16.1	23.8
15-21	1283	49.16	1315	15	11.4	35.2
22-28	518	40.85	537	2	3.7	38.9
More than 28	302	58.53	314			38.9

ported were observed in this small group. Four of the reactions, including the one classified as anaphylactoid, occurred among 19 patients who gave a history of reacting previously to penicillin. The lowest incidence, 13.6/1000, occurred among 590 patients who had previously been treated with penicillin without incident.

The reaction rate of 20.7/1000 patients treated with benzathine penicillin G in oil seems high when compared with the 6.7/1000 observed among venereal disease clinic patients and reported by Smith et al.<sup>2</sup> However, to quote from his paper:

The longer the observation period, the greater is the opportunity for reactions to be observed. It is our opinion, however, that patients reacting are also more apt to return for observation than patients who do not react. This, of course, would result in overly high reaction rates in the longer observation periods. To overcome this bias, the cases were analyzed by duration of planned schedule rather than duration of observation.

For planned schedules of a single session, of two to seven days, of 8 to 14 days, and of over two weeks the reaction rates per 1,000 patients treated were 3, 20, 39, and 56, respectively. It is believed that the rate of 56 per 1,000 approximates the true occurrence of reactions in this series. . . .

The series treated with benzathine penicillin G in oil is a closely followed group, 58 per cent having been observed for more than two weeks and 47 per cent for more than four weeks. The cumulative reaction rate is 26.9/1000 (table II). In spite of the fact that 506 patients were observed for more than two weeks, all the reactions occurred during the first two weeks after treatment.

Although these two studies are not strictly comparable, since the cases reported by Smith et al, principally using PAM, included both single- and multiple-injection therapy, it would appear that benzathine penicillin G in oil is at least as well tolerated as PAM. In a closely observed series, a reaction rate of 2 per cent is not excessive.

#### RESULTS IN THE TREATMENT OF EARLY SYPHILIS

One hundred and seven of the 868 patients were treated for early syphilis and, of these, 89 have been observed for periods ranging from 1 to 18 months (tables III, IV). Fifty-five of the patients observed were treated for primary syphilis, 34

TABLE III

*Results of Benzathine Penicillin G in Oil (1,200,000 Units, One Injection) in the Treatment of Primary and Secondary Syphilis*

Observation period, mo.	Primary syphilis						Secondary syphilis					
	Re-treated			Not re-treated			Re-treated			Not re-treated		
	Cases observed	Num-ber	Per cent	Cumulative per cent	Seropositive Num-ber	Seronegative Per cent	Cases observed	Num-ber	Per cent	Cumulative per cent	Seropositive Num-ber	Seronegative Per cent
1	55				35	63.6	34	20	36.4		33	97.1
2	53				27	50.9	32	26	49.1		30	93.8
3	47				16	34.0	30	31	66.0		27	90.0
4	43	1	2.3	2.3	8	18.6	29	34	79.1		24	82.8
5	39			2.3	3	7.7	27	35	90.0		18	66.7
6	36			2.3	2	5.6	25	33	92.1		16	64.0
7	35			2.3	2	5.7	24	32	91.9		11	45.8
8	34			2.3	2	5.9	24	31	91.7	4.2	8	33.3
9	33			2.3	2	6.1	22	30	91.6	4.2	5	22.8
10	29			2.3	2	7.0	21	26	90.7	9.0	3	14.4
11	25			2.3			20	24	97.7	9.0	3	15.2
12	22			2.3			18	21	97.7	9.0	3	17.1
15	19	1	5.1	7.4			18	18	92.5	9.0	3	17.1
18	14	1	7.1	14.5			12	12	85.4	9.0	2	16.6

Primary syphilis Secondary syphilis	Total re-treated		Reinfection		Clinical relapse	
	3	2	2	1	1	1

TABLE IV

*Results of Benzathine Penicillin G in Oil (1,200,000 Units, One Injection) in the Treatment of Primary and Secondary Syphilis*

Observation period, mo.	Total primary and secondary syphilis							
	Cases observed	Re-treated			Not re-treated			
		Number	Per cent	Cumulative per cent	Seropositive		Seronegative	
					Number	Per cent	Number	Per cent
1	89				68	76.4	21	23.6
2	85				57	67.1	28	32.9
3	77				43	55.8	34	44.2
4	72	1	1.4	1.4	32	44.4	39	54.2
5	66			1.4	21	31.9	44	66.8
6	61			1.4	18	29.6	42	69.0
7	59			1.4	13	22.1	45	76.5
8	58	1	1.7	3.1	10	17.3	46	79.6
9	55			3.1	7	12.8	46	84.1
10	50	1	2.0	5.1	5	10.1	42	84.8
11	44			5.1	3	6.8	39	88.1
12	39			5.1	3	7.7	34	87.2
15	37	1	2.7	7.8	3	8.1	31	84.0
18	26	1	3.8	11.6	2	7.7	21	80.6
Total		5*						

\* Three were reinfected and 2 had a clinical relapse.

for secondary syphilis. During the first year of post-treatment observation, 1 patient with primary syphilis was re-treated for what was considered progression to secondary syphilis, and 2 patients with secondary syphilis were re-treated, 1 for infectious relapse and 1 for reinfection. The cumulative re-treatment rates at the twelfth month are 2.3 per cent for primary syphilis and 9.0 per cent for secondary syphilis. Two additional patients were reinfected, one at 15 months and one at 18 months after treatment for primary syphilis.

For the two stages combined, at 18 months after treatment the cumulative "failure" rate is 3.1 per cent and the cumulative reinfection rate is 8.5 per cent. At this same period 7.7 per cent are still serologically reactive and 80.6 per cent serologically nonreactive. These figures are compared in table V with two other series of patients—one treated with 1,200,000 units of procaine penicillin G in oil with 2 per cent aluminum monostearate (PAM), the other treated with 2,500,000 units of benzathine penicillin G.

These schedules have been weighted by the stages of syphilis in the BOM series; namely, 19 per cent seronegative primary, 43 per cent seropositive primary, and 38 per cent secondary syphilis.

TABLE V

*Comparison of Results 15 to 18 Months after Treatment (Cumulative Percentages)*

	PAM, 1,200,000 units	BOM, 1,200,000 units	Benzathine penicillin G, 2,500,000 units
Re-treated (total)	22.6	11.6	3.6
Serological or clinical relapse	16.5	3.1	1.4
Reinfection	6.1	8.5	2.2
Not re-treated			
Serologically reactive	5.2	7.7	5.1
Serologically nonreactive	72.2	80.6	91.3

The cumulative failure rate (serologic and clinical relapse) after 1,200,000 units of PAM was 16.5 per cent, after 2,500,000 units of benzathine penicillin G, 1.4 per cent. The reinfection rate ranged from 2.2 to 8.5 per cent among the three schedules. Since reinfection is not easily differentiated from clinical relapse or progression, the total cumulative re-treatment rate is also shown. This rate, which may be considered the maximum failure rate at 18 months after treatment, is 22.6 per cent for PAM, 11.6 per cent for BOM, and 3.6 per cent for benzathine penicillin G. In terms of satisfactory results, the seronegativity (or nonreactive) rates for the three schedules are 72, 81, and 91 per cent, respectively.

Although the number of cases included in the evaluation is small, the results indicate that BOM is more effective than PAM in the same dosage, but less effective than benzathine penicillin G administered in the recommended dosage.

#### BIBLIOGRAPHY

1. SMITH, C. A., AND PRICE, E. V.: American experience with penicillin alone in the treatment of syphilis, *World Health Organ./VDT*, p. 228, July, 1957.
2. SMITH, C. A.; CUTLER, J. C., AND PRICE, E. V.: Penicillin reactions in a venereal disease clinic population. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 144.

# Combined Gamma Globulin and Chloramphenicol Therapy of Lymphogranuloma Venereum

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Host resistance in infectious diseases, whether they are caused by bacteria, viruses, treponemas, protozoans, or even helminths, usually is more profoundly influenced by environmental and anthropological factors in the tropics than in temperate climates.<sup>1</sup> Some infections, because of certain natural, racial, and acquired immunities, seldom become severe in tropical indigenes, while others, when they are affected adversely by the aforesaid "nonspecific" factors, will advance more rapidly to their late stages than otherwise might be supposed possible. Also, prolonged coexistence of infection and fluctuating immunity is more peculiar to tropical climates. When infections are long-standing or have progressed rapidly to their late stages, it can be supposed that a point has been reached at which the pituitary-adrenal axis becomes exhausted, and, when complicated by protein and certain vitamin deficiencies, the resistance of the host must be depressed further. It is during such periods that infections, due to agents that are ordinarily susceptible to antibiotics, may be found to be unaffected by them.

Since depressed host resistance, undoubtedly influenced by and coupled with some of these "nonmicrobial determinants of infectious disease" (Dubos), could account for such therapeutic failures, the apparent unresponsiveness of the infection in these circumstances must not be considered merely as an instance of increased resistance of the pathogenic agent to the antibiotic. Thus, when the host cannot contribute materially to his own defenses, it is evident that more than antibiotic therapy alone will be required to effect a cure.

Of particular interest in the development of a combined immunological and antimicrobial approach to the treatment of severe infections (perplexed by the variance of reports on the comparative efficacy of sulfonamides, specific immune serum, and their combination in the treatment of pneumococcal infections in rats), we set out to investigate the use of these therapeutic agents singly and in combination in similar infections, in 1940.<sup>2</sup> These latter experiments differed from those that had been reported previously in that: (1) we included only proved bacteremic pneumococcal infections in the analysis of the results of treatment; (2) we used a technique for inducing pneumococcal infections with mouse peritoneal exudate that was not traumatizing;<sup>3</sup> and (3) we compared combined half doses of the sulfonamide and specific rabbit antipneumococcal serum with what we considered were optimal doses of each. In type I pneumococcal infections of rats (in fatal instances, the majority had pneumonia and expected characteristic pyogenic complications), it was demonstrated that although the resistance of the host had been intensely challenged by overwhelming infection, it could be reinforced effectively and successfully by the administration of combined half doses of sulfapyridine and specific immune serum as well as by optimal or curative doses of only immune serum. In such infections, we found that sulfapyridine alone cured only about 40 per cent of the animals, while combined therapy was successful in twice as many.

Similar experiments, in which we used sulfadiazine or dimethylsulfadiazine combined with specific immune rabbit serum, confirmed the foregoing.<sup>4</sup> The results of these experiments consequently were put into practice by treating severe pneumococcal and staphylococcal infections in human beings with a combination of specific

immune serum and sulfadiazine.<sup>5</sup> The results of such combined immunological and antimicrobial therapy of human infections were comparable to those obtained in animals treated similarly.

Recently, a novel immunological and antimicrobial approach to reliably augmenting host resistance in bacterial infections was reported by Fisher<sup>6</sup> who successfully treated experimental bacterial infections in mice with subcurative doses of pooled human gamma globulin and chloramphenicol. Their observations on this immunological antibiotic combination were confirmed subsequently by Welch<sup>7</sup> in the treatment of hemolytic streptococcal infections of mice.

In 1954, 6 young children, who were thought to have disturbed immune mechanisms that caused them to be refractory to antibiotic therapy, were treated successfully with human gamma globulin and an antibiotic by Harris and Schick.<sup>8</sup> In 1957, Waisbren<sup>9</sup> reported that a combination of pooled human gamma globulin and chloramphenicol could provide to adults the expedients for recovery from infections that had not been responsive to just antibiotics. The results that he obtained with this combination were remarkable in a number of cases, in which, as a result of infection, the illness had progressed to such a stage that a hopeless outcome only could be expected. Although Waisbren considered the possibility of properdin or other bactericidal substances having been administered in the pooled human gamma globulin as an interesting explanation for the apparent effectiveness of this combined therapy, he noted that there was as yet no proof that such was the case, nor were any other definitive answers readily available.

It is known that pooled human gamma globulin fractions contain an indefinite number of antibodies to infectious agents pathogenic for man. The nature of this antibody content depends largely on the diversity of infectious diseases that the sources of the pooled supplies have experienced or encountered. It is appreciated that some of the infectious disease components of this antibody complex may have been stimulated by diverse antigens without the development of manifest disease. Specific antibodies to certain infections may not be detectable, and whether this is due to their complete absence or to faulty technique cannot be resolved presently. On the other hand, although specific antibodies to certain infectious agents may not be found, cross-reacting antibodies to closely related infections may be present. There are no euglobulins, including properdin, in pooled human gamma globulin.

These and other peculiarities of pooled human gamma globulin were recognized when it was proposed to study the efficacy of the combination of pooled human gamma globulin and chloramphenicol in the treatment of certain tropical diseases. It was realized also, because of the limiting conditions imposed by the locality of the study, some infections would not be available for treatment. We recognized that the advantage of such combined therapy in many instances, particularly in prevalent acute or early stages of some infections, seemed to be limited by the availability as well as by the cost of gamma globulin, and the fact that many of these infections were responsive to antibiotic therapy alone. Thus vis-a-vis with these practical considerations, we decided to restrict the early phases of this study to the treatment of several infectious diseases, including lymphogranuloma venereum, late yaws, tropical ulcer, and chronic brucellosis. The results of treatment of lymphogranuloma venereum with the combination will be reported in this paper.

#### DOSAGE

Before beginning the studies on the treatment of any of these diseases, we thought that it would be expedient to find out if the combination were more effective than

the antibiotic alone and if smaller doses of chloramphenicol would be effective when given in combination with gamma globulin.

Although we had treated many cases of lymphogranuloma venereum with antibiotics, chloramphenicol included, and knew what responses to expect from such treatment in advanced secondary as well as in tertiary cases, we realized that discretionary dosages of chloramphenicol and gamma globulin would have to be devised. Thus, the dosage of chloramphenicol was set at 1 Gm. daily for seven days for treating the secondary lesions and 1 Gm. daily for 14 days for treating late or tertiary lesions. In those patients with secondary lesions, it was decided to inject 10 ml. of gamma globulin initially and to repeat the injection four days later if it was thought to be necessary. In patients with late or tertiary lesions, it was decided to inject 10 ml. of gamma globulin and to repeat such injections at intervals of four days, when the clinical responses to chloramphenicol and each previous dose of gamma globulin could be evaluated and the necessity of further dosage determined.

THERAPEUTIC RESULTS

Eleven patients with advanced secondary lesions, including abscessed and indurated inguinal buboes, and pelvic and rectal syndromes, and 10 patients with late or tertiary lesions, including urethral stricture, elephantiasis, and vulvar ulceration accompanying esthiomene, were treated with the combination. The clinical diagnosis of lymphogranuloma venereum was confirmed by the intradermal test (Frei) and/or the complement fixation test. The duration of infection in the earliest case was longer than six months. Hyperglobulinemia was noted with regularity in cases in which the duration of infection was less than one year (table I).

In 3 of the 11 patients with advanced secondary lesions of lymphogranuloma venereum, it was decided on the fourth day of therapy that a single dose of 10 ml. of gamma globulin was sufficient. In the other 8, however, because of more extensive involvement or slower clinical responses, a second injection of 10 ml. of gamma globulin was considered to be necessary. The adequacy of this discretionary dosage of gamma globulin combined with chloramphenicol was borne out by the following: in none of these cases did we find it necessary to incise or drain abscessed lymph nodes; additional courses of therapy were not required; and no relapses were observed during periods of 4 to 12 months' post-treatment.

TABLE I  
Summary of Cases

Cases, sex and age	Lesions	Dosages	Remarks
M, 21; M, 27; M, 25; M, 22; M, 21; M, 29; M, 35; F, 47; M, 35; M, 42	M, 44; M, 47; M, 23; F, 20; M, 30; M, 40; M, 26; M, 38;	Advanced 2°. Inguinal (buboes suppurated or indurated), pelvic (adenitis), and rectal (proctitis) syndromes	Gamma globulin, 1 or 2 to 10 ml. intramuscular doses Chloramphenicol, 7 to 1 Gm. daily oral doses
M, 21; M, 29; M, 35; F, 47; M, 35; M, 42	M, 44; M, 47; M, 23; F, 20; M, 30; M, 40; M, 26; M, 38;	Late. Tertiary. Chronic inguinal and rectal syndromes (urethral and rectal strictures; urethral abscesses; urethral, scrotal fistulae)	Gamma globulin, 2 to 10 ml. intramuscular doses Chloramphenicol, 14 to 1 Gm. daily oral doses
F, 28; F, 20	Chronic pelvic and anogenital syndromes (vulvar and anal papillomata, esthiomene, vulvar ulceration)	Gamma globulin 3 and 4 to 10 ml. intramuscular doses Chloramphenicol, 14 to 1 Gm. daily oral doses	Regression of lesions satisfactory in all cases; 8 patients required 2 doses of gamma globulin; no surgery was necessary; no relapses during 4 to 12 mo. post-treatment
M, 25	Chronic inguinal syndrome (elephantiasis of penis and scrotum; latter, 60 cm. circumference by 25 cm. long)	Gamma globulin 6 to 10 ml. intramuscular doses Chloramphenicol, 2 courses of 14 to 1 Gm. daily oral doses	Regression of lesions satisfactory in all cases; urethral and rectal dilatations in cases with strictures°
			Regression of papillomas and esthiomene by four to five weeks post-treatment; healing of ulcerations by one week post-treatment°
			Regression of elephantiasis to approximately 1/3 of stated size within period of therapy; surgical removal of remaining tissues°

° No relapses were observed in late or chronic cases during 7 to 13 months post-treatment.

In cases with late or tertiary lesions of lymphogranuloma venereum, also using an arbitrary dosage, we found that only two injections of 10 ml. of gamma globulin were required in 7 of the 10 patients, while in 3 others three to six doses were needed. Two of these requiring three doses had vulvar esthiomene with ulceration, while the third was a case of massive elephantiasis of the scrotum and penis, in which six doses were given. The patients with tertiary lymphogranuloma venereum lesions have been observed for periods of 7 to 13 months' post-treatment, and as yet no relapses have occurred.

An interesting clinical reaction was noticed after the earlier injections of gamma globulin—a manifest alteration of the mood and the replacement of commonly observed depression by a euphoric sense of well-being.

Having compared these results on the use of the combination with those on the use of the antibiotic alone in the treatment of lymphogranuloma venereum, we believe that: (1) considerably shorter courses of therapy using smaller doses of the antibiotic are possible when chloramphenicol is given in combination with gamma globulin; and (2) the combination is definitely more effective than chloramphenicol alone or, for that matter, any antibiotic administered singly in the treatment of advanced secondary and tertiary lesions of lymphogranuloma venereum.

Before attempting to develop a rationale for the biological mechanisms responsible for the apparent efficacy of the combination, it would seem that we should be apprised of certain characteristics of the virus, as well as of some of the immunological aspects of the disease that it causes.

The virus, *Miyagawanella lymphogranulomatis*, like that of psittacosis, is one of the large viruses of the family Chlamydiaceae that range in size from about 300 to 450 m $\mu$ . Because of their relatively large size and their susceptibility in vitro to certain antibiotics and sulfonamides, the viruses of this group bear some resemblance to rickettsiae and smaller bacteria. Unlike the rickettsiae, however, these viruses do not require an insect vector to maintain the disease cycle, while their dependency in vitro on host-cell materials and physiological systems for reproduction, their ability to invade susceptible cells, and to multiply and develop basophilic inclusion bodies within these cells distinguish them from the smaller bacteria.

Lymphogranuloma venereum—a world-wide venereal disease characterized by disabling complications in its late stages—is ranked only below syphilis and gonorrhea as a public health problem. On the basis of complement fixation tests, the members of the psittacosis-lymphogranuloma venereum group of viral diseases may be antigenically related. Neutralization tests and the lack of cross immunity in susceptible animals, however, indicate individual antigenic differences. Generally speaking, serum antibodies—physically indistinguishable from gamma globulins—develop on recovery from viral infections, whether the illness has been apparent or not. Among these antibodies are protective, agglutinating, complement-fixing, and neutralizing antibodies. While the complement-fixing antibodies are not concerned with protective immunization, the neutralizing antibodies, although the mechanism is not fully known, probably coat the virus particles in a loose combination that prevents them from attaching to the receptors of new susceptible cells and invading them. Although this process can lead indirectly to the death of the virus, it is unlikely that the infectivity of all or even of many of the virus particles acted upon by such antibodies is actually destroyed. Evidence points to inactivation of the virus when it is extracellular, since, when it has penetrated the cell, it seems to be adequately protected from antibodies and antiviral agents. Although reports on circulating neutralizing antibodies in lymphogranuloma venereum are contradictory, it does

seem probable that faulty techniques, such as too short incubation or the use of too concentrated viral suspensions, may account for negative results. Also, in lymphogranuloma venereum, low titers of circulating antibodies may be explained partly by the existence of "sessile" antibodies that are found characteristically in the infected tissues.

At this point, since a number of nonspecific factors apparently were influencing unfavorably the ability of these patients with chronic lymphogranuloma venereum to form adequate amounts of antibody, we might wonder if: (1) the administration of pooled human gamma globulin might have supplied specifically modified globulin antibody due to previous infections among the donors of the pooled gamma globulin; (2) pooled human gamma globulin might have supplied cross-reacting antibodies, resulting from previous infections with other members of this family of viruses; (3) the effectiveness of these combinations of gamma globulin and chloramphenicol might have been due to properdin being administered in this solution of pooled human gamma globulin, as suggested by some investigators; and (4) the effectiveness of these combinations of gamma globulin and chloramphenicol might be attributed to the administration of a fresh supply of gamma globulin that was susceptible of modification to so-called "sessile" antibody at the site of infection or to other specific antibodies at the usual sites of production of modified globulin antibody.

Accordingly, on the basis of previously acquired information as well as conjecture, we believe that: (1) since the incidence of this infection probably is very low in the usual donor pools of gamma globulin, it is doubtful that we could have supplied enough antibody to supplement adequately the action of the antibiotic in producing the favorable clinical responses in these cases; (2) it is doubtful also that sufficient cross-reacting antibodies could have been supplied, even though a number of donors of the pooled gamma globulin could have had previous infections with other members of the family Chlamydiaceae; (3) as noted before, properdin—a euglobulin with a molecular weight eight times that of gamma globulin—would not be included in this solution of gamma globulin antibodies and thus was not administered; (4) the administration of pooled human gamma globulin could have provided a fresh source of gamma globulin that was susceptible of modification to the "sessile" antibodies characteristic of this infection, particularly since local tissue immunological mechanisms seem to be highly important in coping with this virus; or (5) other specific antibodies could have been formed from the freshly supplied gamma globulin at the usual sites of formation of modified globulin antibody.

#### BIBLIOGRAPHY

1. LOUGHLIN, E. H., AND MULLIN, W. G.: Certain aspects of deficiency diseases of the tropics and treatment of some related infections, *Ann. New York Acad. Sc.* 63:276, 1955.
2. SPITZ, S. H.; LOUGHLIN, E. H., AND BENNETT, R. H.: Treatment of pneumonia in rats, *J. Lab. & Clin. Med.* 26:1284, 1941.
3. LOUGHLIN, E. H.; SPITZ, S. H., AND BENNETT, R. H.: A simple nontraumatizing method of inducing pneumococcal infection in albino rats, *J. Lab. & Clin. Med.* 26:1181, 1941.
4. LOUGHLIN, E. H.; BENNETT, R. H.; FLANAGAN, M. E., AND SPITZ, S. H.: Treatment of experimentally induced type I pneumococcus pneumonia in albino rats, *J. Lab. & Clin. Med.* 28:1455, 1943.
5. LOUGHLIN, E. H.: Unpublished observations.
6. FISHER, M. W.: Synergism between human gamma globulin and chloramphenicol in the treatment of experimental bacterial infections, *Antib. & Chemo.* 7:315, 1957.
7. WELCH, H.: (Editorial) The host and the parasite. A new clinical approach to biologic relationships, *Antib. & Chemo.* 7:271, 1957.
8. HARRIS, J. R., AND SCHICK, B.: The use of gamma globulin in infection refractory to antibiotics, *J. Mt. Sinai Hosp.* 21:148, 1954.
9. WAISBREN, B. A.: The treatment of bacterial infections with the combination of antibiotics and gamma globulin, *Antib. & Chemo.* 7:322, 1957.

In the present study four different penicillin V preparations were given orally to healthy human beings, and the uptake and excretion were followed by means of penicillin determinations in plasma and urine. The purpose was to provide data for a correct relative evaluation of different forms of oral penicillin V, in addition to those supplied by other investigators.<sup>3-12</sup>

## MATERIAL

Since the gastrointestinal absorption of penicillin V is influenced both by the solubility of the compound used and by the stomach filling, both these factors were varied. Three compounds were used: (1) the sparingly soluble free acid of penicillin V (solubility approximately 0.05 per cent in water at room temperature), (2) the freely soluble potassium salt, and (3) the calcium salt, which has a medium solubility (approximately 1.4 per cent in water at room temperature).

As the penicillin V acid might be dissolved and absorbed at a different rate, depending on the crystal size, both large and fine crystals (100 to 200  $\mu$  and 5 to 10  $\mu$ , respectively) of the acid were tested. Thus, in all, four preparations were used.

All compounds were given as rapidly disintegrating tablets, each tablet containing 250,000 units of penicillin V (equivalent to 150 mg. of penicillin V acid). One tablet per dose was used.

Disintegration time of tablets: Penicillin V acid, large crystals (100 to 200  $\mu$ ),  $\frac{1}{2}$  minute; penicillin V acid, fine crystals (5 to 10  $\mu$ ), 1 minute; potassium penicillin V, 2 min.; calcium penicillin V,  $\frac{1}{2}$  minute.

Each of the four preparations were given both on a fasting stomach and after a standardized meal, which makes a total of eight test series.

Benzathine penicillin V was not included in the series, because it has been thoroughly studied by various authors.<sup>3,5,6,9,12</sup> It gives plasma levels similar to or somewhat lower than those obtained from the acid; its main advantage is its suitability as an ingredient in stable aqueous suspensions.

## TEST SUBJECTS

For these studies 12 healthy nurses were used, all subjects being represented in all series, thus securing a complete crossover study.

Healthy subjects were used, because it was felt that any differences due to different acid stability of the preparations would be detected more easily in this way. (It is known, for example, that an acidified solution of potassium penicillin V is considerably less stable in vitro than an aqueous suspension of penicillin V acid with the same pH.<sup>1</sup>) All subjects were found to have free gastric acid (test with Diagnex blue); no quantitative acidity control was made.

When given after a meal the tablets were administered 30 minutes after a standardized breakfast consisting of two sandwiches with cheese, one boiled egg, one glass of milk, and one cup of black coffee.

TABLE I

*Pencillin V Acid, 250,000 Units, Large Crystals (100 to 200  $\mu$ ), Given to Fasting Subjects*

Subject no.	Penicillin in plasma, units/ml., hr. after dose							Penicillin in urine, units		
	0	½	1	2	4	6	8	0 to 4 hr.	4 to 8 hr.	Total
1	0.016	0.88	2.0	0.60	0.036	0	0	55,700	1,400	57,100
2	0.025	0.88	2.6	1.52	0.144	0.057	0	52,500	5,100	57,600
3	0	0.34	1.45	0.48	0.040	0	0	44,500	1,300	45,800
4	0	1.70	4.4	0.48	0.033	0	0	43,200	800	44,000
5	0.038	0.123	0.150	0.141	0.20	0.22	0.053	90,700	10,500	101,200
6	0.032	2.0	1.00	0.25	0.039	0.033	0.030	45,000	1,100	46,100
7	0.025	1.40	2.7	0.43	0.090	0.050	0.039	5,600	3,700	9,300
8	0	0.52	0.37	0.36	0.165	0.100	0.052	75,000	11,500	86,500
9	0	1.80	2.7	0.50	0.050	0	0	57,500	1,100	58,600
10	0	2.0	1.56	0.49	0.097	0.025	0	22,500	4,300	26,800
11	0	2.1	1.50	0.46	0.053	0.025	0	85,000	1,300	86,300
12	0	1.50	3.4	0.38	0.058	0	0	38,600	1,600	40,200
Average	0.011	1.27	1.99	0.51	0.084	0.043	0.015	51,320	3,640	54,960
Per cent								20.5	1.5	22.0

TABLE II

*Pencillin V Acid, 250,000 Units, Small Crystals (5 to 10  $\mu$ ), Given to Fasting Subjects*

Subject no.	Penicillin in plasma, units/ml., hr. after dose							Penicillin in urine, units		
	0	½	1	2	4	6	8	0 to 4 hr.	4 to 8 hr.	Total
1	0	0.84	3.2	0.66	0.145	0.060	0.041	58,500	7,800	66,300
2	0.025	0.198	1.04	1.32	0.44	0.082	0.034	50,000	6,700	56,700
3	0	0.30	0.64	1.20	0.75	0.033	0	42,300	5,600	47,900
4	0	1.35	2.6	0.53	0.042	0	0	44,000	600	44,600
5	0.025	2.6	2.9	0.65	0.104	0.037	0.025	41,800	4,200	46,000
6	0	2.2	2.3	0.69	0.062	0.045	0.025	36,900	3,800	40,700
7	0	2.1	1.88	0.45	0.050	0.045	0.037	55,000	2,200	57,200
8	0.025	1.00	1.06	0.27	0.056	0.025	0	30,900	8,700	39,600
9	0	1.90	1.10	0.73	0.180	0.025	0	62,500	7,200	69,700
10	0	2.0	4.2	1.10	0.050	0.025	0	43,300	5,600	48,900
11	0	0.26	0.50	1.10	0.082	0.048	0	87,400	3,800	91,200
12	0	0.25	0.95	1.05	0.20	0.025	0	75,600	5,800	81,400
Average	0.006	1.25	1.86	0.81	0.180	0.038	0.014	52,350	5,170	57,520
Per cent								20.9	2.1	23.0

TABLE III

*Pencillin V Potassium, 250,000 Units, Given to Fasting Subjects*

Subject no.	Penicillin in plasma, units/ml., hr. after dose							Penicillin in urine, units		
	0	½	1	2	4	6	8	0 to 4 hr.	4 to 8 hr.	Total
1	0.034	6.2	2.5	0.59	0.044	0.034	0	72,900	900	73,800
2	0.041	4.8	1.84	1.30	0.150	0.045	0.025	78,800	2,600	81,400
3	0	7.0	4.6	1.55	0.071	0.025	0	64,400	1,300	65,700
4	0.025	5.0	2.7	0.50	0.055	0.032	0.032	66,300	900	67,200
5	0	4.0	1.36	0.96	0.050	0.040	0	74,400	1,500	75,900
6	0	3.2	1.48	1.35	0.042	0.025	0	90,000	3,400	93,400
7	0	4.8	2.7	0.36	0.046	0	0	96,000	800	96,800
8	0.025	2.6	1.60	0.55	0.041	0.025	0	62,500	600	63,100
9	0	2.2	2.0	0.50	0.048	0	0	72,500	1,200	73,700
10	0	3.9	2.0	0.62	0.056	0.040	0	101,200	4,500	105,700
11	0	4.7	1.92	0.65	0.050	0	0	103,500	1,200	104,700
12	0	4.7	4.0	0.62	0.062	0.038	0	108,000	2,300	110,300
Average	0.010	4.4	2.4	0.80	0.060	0.025	0.005	82,540	1,770	84,310
Per cent								33.0	0.7	33.7

TABLE IV  
*Penicillin V Calcium, 250,000 Units, Given to Fasting Subjects*

Subject no.	Penicillin in plasma, units/ml., hr. after dose							Penicillin in urine, units		
	0	½	1	2	4	6	8	0 to 4 hr.	4 to 8 hr.	Total
1	0.025	4.3	3.0	0.41	0.048	0.025	0	67,200	200	67,400
2	0.024	1.15	1.20	1.15	0.088	0.041	0	56,700	1,400	58,100
3	0.050	4.4	2.6	0.39	0.076	0.046	0.025	115,500	1,300	116,800
4	0	3.5	1.50	0.33	0.044	0.043	0.040	68,900	900	69,800
5	0.025	1.72	—	0.63	0.050	0.046	0.025	74,300	2,600	76,900
6	0.025	2.5	1.90	0.33	0.043	0.025	0	71,400	2,300	73,700
7	0	3.0	1.90	0.38	0.050	0.025	0	78,400	900	79,300
8	0	2.4	1.70	0.30	0.100	0.037	0	80,800	3,300	84,100
9	0	4.2	2.7	0.60	0.056	0.025	0	81,600	2,700	84,300
10	0	1.44	1.90	0.63	0.21	0.059	0.042	72,500	9,200	81,700
11	0	1.00	1.25	0.63	0.125	0.030	0	74,900	7,000	81,900
12	0	4.6	2.4	0.57	0.063	0	0	90,000	2,500	92,500
Average	0.012	2.9	2.0	0.53	0.079	0.034	0.011	77,680	2,860	80,540
Per cent								31.0	1.1	32.1

TABLE V  
*Penicillin V Acid, 250,000 Units, Large Crystals (100 to 200  $\mu$ ), Given After a Standardized Meal*

Subject no.	Penicillin in plasma, units/ml., hr. after dose							Penicillin in urine, units		
	0	½	1	2	4	6	8	0 to 4 hr.	4 to 8 hr.	Total
1	—	0.027	0.37	0.95	0.23	0.028	0	66,000	8,200	74,200
2	—	0.150	0.48	0.88	0.28	0.043	0.025	46,200	8,800	55,000
3	—	0.28	0.65	0.45	0.145	0.034	0.025	59,400	4,200	63,600
4	—	0.28	0.48	0.43	0.145	0.060	0.054	31,900	8,400	40,300
5	—	0.45	0.88	0.68	0.20	0.056	0.025	46,200	8,400	54,600
6	—	0.25	0.58	1.00	0.30	0.050	0.025	53,100	14,800	67,900
7	—	0.061	0.23	0.170	0.39	0.24	0.175	18,500	21,300	39,800
8	—	0.165	0.50	0.39	0.25	0.105	0.063	43,200	13,800	57,000
9	—	1.60	1.15	0.80	0.082	0.025	0	83,200	2,000	85,200
10	—	0.44	2.1	0.78	0.180	0.052	0.040	77,400	9,700	87,100
11	—	0.061	0.20	0.32	0.60	0.125	0.115	47,500	29,200	76,700
12	—	0.24	0.50	2.0	0.24	0.080	0.050	69,800	9,500	79,300
Average	—	0.33	0.68	0.74	0.25	0.075	0.050	53,480	11,550	65,030
Per cent								21.4	4.6	26.0

TABLE VI  
*Penicillin V Acid, 250,000 Units, Small Crystals (5 to 10  $\mu$ ), Given After a Standardized Meal*

Subject no.	Penicillin in plasma, units/ml., hr. after dose							Penicillin in urine, units		
	0	½	1	2	4	6	8	0 to 4 hr.	4 to 8 hr.	Total
1	—	0.100	0.39	0.34	0.45	0.20	0.165	30,200	13,900	44,100
2	—	0.145	0.85	1.45	0.25	0.046	0.025	90,300	9,900	100,200
3	—	1.80	1.60	0.73	0.061	0.025	0	75,600	1,500	77,100
4	—	0.074	0.65	0.88	0.155	0.057	0.025	76,700	10,100	86,800
5	—	0.080	0.25	0.25	0.50	0.140	0.110	34,800	24,000	58,800
6	—	0.064	0.35	1.05	1.00	0.100	0.045	38,500	10,600	49,100
7	—	0.24	0.98	0.55	0.092	0.040	0.035	41,000	2,700	43,700
8	—	0.26	0.85	0.80	0.145	0.040	0.035	43,700	3,900	47,600
9	—	0.061	0.93	1.00	0.100	0.025	0	49,600	3,000	52,600
10	—	0.115	0.35	0.78	0.120	0.086	0.045	41,300	10,500	51,800
11	—	0.22	0.83	0.55	0.110	0.042	0.025	43,700	5,200	48,900
12	—	0.072	0.29	0.65	0.20	0.039	0.025	29,600	7,900	37,500
Average	—	0.27	0.69	0.75	0.27	0.070	0.045	49,580	8,600	58,180
Per cent								19.8	3.4	23.3

TABLE VII

*Penicillin V Potassium, 250,000 Units, Given after a Standardized Meal*

Subject no.	Penicillin in plasma, units/ml., hr. after dose							Penicillin in urine, units		
	0	½	1	2	4	6	8	0 to 4 hr.	4 to 8 hr.	Total
1	—	0.70	1.26	0.70	0.066	0.045	0.025	38,900	2,700	41,600
2	—	0.80	1.44	0.75	0.155	0.047	0.025	55,200	5,100	60,300
3	—	0.31	0.58	0.70	0.155	0.045	0	36,700	5,800	42,500
4	—	0.48	0.36	0.60	0.125	0.071	0.050	22,400	7,400	29,800
5	—	0.52	1.00	0.63	0.079	0.048	0.025	28,600	4,600	33,200
6	—	0.72	0.60	1.00	0.070	0	0	40,800	6,300	47,100
7	—	1.05	1.00	0.73	0.100	0.064	0.089	50,400	4,400	54,800
8	—	0.83	0.53	0.68	0.21	0.145	0.055	81,000	13,100	94,100
9	—	1.05	1.45	1.10	0.089	0.025	0	96,600	3,700	100,300
10	—	0.73	0.88	0.90	0.155	0.050	0.048	90,000	6,900	96,900
11	—	0.29	0.58	0.43	0.32	0.049	0	48,500	7,400	55,900
12	—	0.60	0.78	0.60	0.25	0.076	0.092	36,500	7,300	43,800
Average	—	0.67	0.87	0.74	0.148	0.055	0.034	52,130	6,230	58,360
Per cent								20.9	2.5	23.3

## PENICILLIN DETERMINATIONS

Penicillin V plasma levels were determined one-half, one, two, four, six, and eight hours after the dose.

Urinary recovery of penicillin V was determined in two portions of urine, one collected during the first four hours after the dose and the other during the next four hour period.

The determinations were carried out with the agar plate technique, with a strain of *Sarcina lutea* used as test organism. Values below 0.025 units/ml. are not considered as fully reliable with the method used.

## RESULTS

The results are shown in tables I through VIII and figures 1 to 3. (Figures 1 and 2 show the same curves in different arrangements.) A statistical analysis of the findings has shown the following facts.

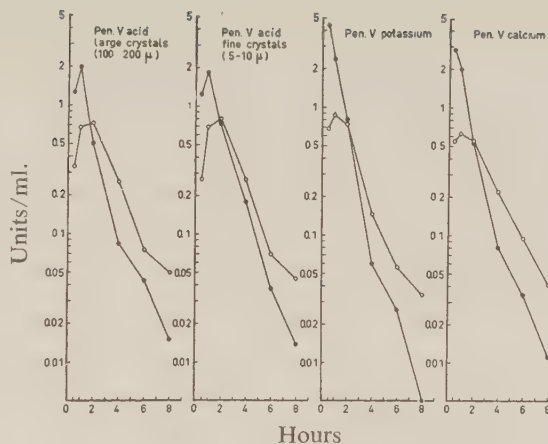
No difference whatever has been found between large and fine crystals of penicillin V acid. Thus the crystal size has no influence on the uptake. In the further statistical treatment the two penicillin V acid groups have been combined to one.

TABLE VIII

*Penicillin V Calcium, 250,000 Units, Given after a Standardized Meal*

Subject no.	Penicillin in plasma, units/ml., hr. after dose							Penicillin in urine, units		
	0	½	1	2	4	6	8	0 to 4 hr.	4 to 8 hr.	Total
1	—	0.51	0.68	0.48	0.042	0.025	0	38,000	500	38,500
2	—	0.46	0.78	0.54	0.094	0.035	0	45,000	800	45,800
3	—	0.26	0.50	0.47	0.190	0.044	0	39,500	6,600	46,100
4	—	0.120	0.21	0.23	0.155	0.050	0.041	28,000	10,600	38,600
5	—	0.33	0.57	0.69	0.092	0.045	0.025	58,800	4,500	63,300
6	—	0.24	0.29	0.60	0.145	0.034	0.025	48,000	8,000	56,000
7	—	0.77	0.55	0.45	0.42	0.36	0.086	37,700	24,500	62,200
8	—	0.105	0.135	0.46	0.22	0.180	0.170	13,800	17,500	31,300
9	—	1.35	1.80	1.02	0.080	0.025	0	81,600	3,200	84,800
10	—	1.90	1.40	0.60	0.105	0.056	0.025	91,800	7,700	99,500
11	—	0.054	0.105	0.29	0.58	0.160	0.050	57,600	20,700	78,300
12	—	0.50	0.50	0.73	0.50	0.115	0.063	56,700	18,500	75,200
Average	—	0.55	0.63	0.55	0.22	0.094	0.040	49,710	10,260	59,970
Per cent								19.9	4.1	24.0

FIG. 1. Plasma penicillin levels after 250,000 units of penicillin V in different forms, given as rapidly disintegrating tablets, are shown. The same 12 healthy persons were used in a crossover study. Average values are given. •—• fasting; o—o after a standard meal.



In general no significant differences have been found between the potassium and calcium salts of penicillin V. Only as far as plasma peak values on a fasting stomach are concerned, a difference was found (on the  $P < 0.05$  significance level), in favor of the potassium salt. Consequently, these two groups have in most cases also been combined into one.

It is evident from figure 1 that for all preparations tested plasma peaks are lower but duration longer, when the tablets are given after a meal than on a fasting stomach. The difference in duration amounts to over one hour. Limit for duration was then set at 0.05 units/ml.

A comparison of the plasma levels obtained from the different preparations (fig. 2) shows much less pronounced differences. After meal no significant differences were found at all. On a fasting stomach only one difference is of high significance, viz., the peak from the potassium salt is definitely higher than that from the acid ( $P < 0.001$ ). The difference in peak potassium to calcium is significant only on the lowest level, and any difference for calcium to acid is not significant.

Both on a fasting stomach and after meal there seems to be a tendency for the potassium salt to give a slightly shorter duration; in the present material the difference is not statistically significant, however. This point will be further discussed.

One point of interest should be stressed regarding the values for urinary recovery: With few exceptions the 0 to 4 hour urine values reflect the plasma peaks, since differences in urine values are generally significant or not significant parallel

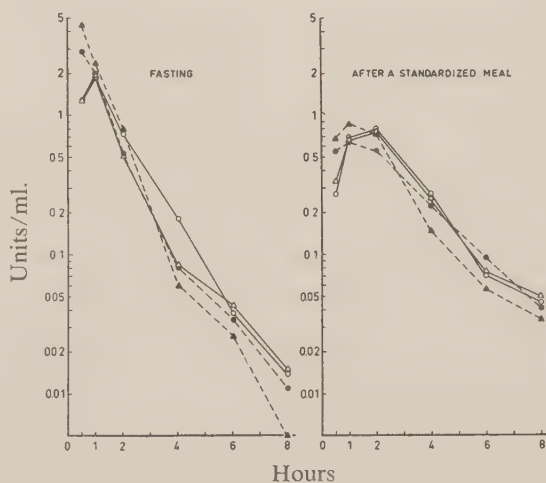


FIG. 2. Plasma penicillin levels after 250,000 units of penicillin V in different forms, given as rapidly disintegrating tablets, are shown. The same 12 healthy persons were used in a crossover study. Average values are given.  $\triangle$ — $\triangle$  Penicillin V acid, large crystals (100 to 200  $\mu$ );  $\blacktriangle$ — $\blacktriangle$  penicillin V potassium; o—o penicillin V acid, fine crystals (5 to 10  $\mu$ );  $\bullet$ — $\bullet$  penicillin V calcium.

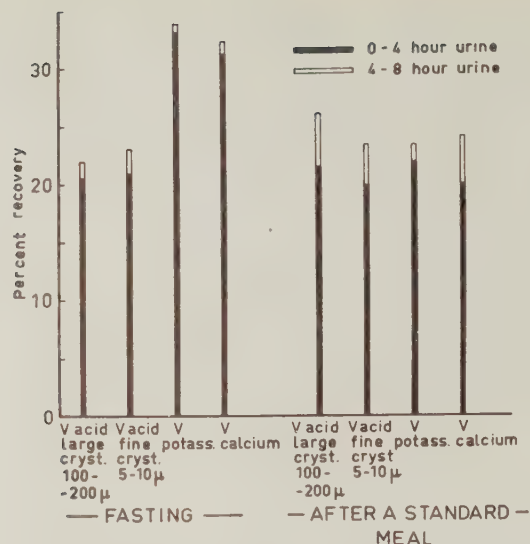


FIG. 3. Penicillin recovery in urine after 250,000 units of penicillin V in different forms, given as rapidly disintegrating tablets, is illustrated. The same 12 healthy persons were used in a crossover study. Average values are shown.

with the corresponding differences in plasma peaks. A similar conformity also generally applies to the 4 to 8 hour urine values and the duration in plasma. Some of these findings will be further discussed.

The statistical analysis is summarized in tables IX to XII. Figures for penicillin in plasma and urine are geometrical mean values; "acid" refers to the two groups "large" and "fine" crystals combined, "salt" to potassium and calcium salts combined. Values for acid are always combined, since in no case were the differences found due to crystal size.

#### DISCUSSION

The higher peaks obtained by penicillin V preparations on a fasting stomach than after a meal have been found by several other investigators;<sup>4, 6-8</sup> this fact has caused a recommendation to give penicillin V preferably on a fasting stomach.<sup>4, 6</sup> No previous investigators, however, with one recent exception,<sup>11</sup> seem to have given attention to the significantly longer duration obtained when the preparations are given after a meal. This difference in duration apparently can be found also in one

TABLE IX  
Statistical Analysis: Plasma Peak Values (p)

Item	Value (geom. mean)	Range (95 per cent confidence)	Significance for ratio $\neq 1$
<b>Fasting</b>			
$p_{\text{acid}}$	1.81 u./ml.		
$p_{\text{potassium}}$	4.20 u./ml.		
$p_{\text{calcium}}$	2.64 u./ml.		
Ratio $p_{\text{potassium}}/p_{\text{acid}}$	2.3	(1.6-3.4)	$P < 0.001$
Ratio $p_{\text{potassium}}/p_{\text{calcium}}$	1.6	(1.0-2.5)	$P < 0.05$
Ratio $p_{\text{calcium}}/p_{\text{acid}}$			Not significant
<b>After Meal</b>			
$p$	0.81 u./ml., all preparations combined		No significant difference between preparations
<b>Ratio: Fasting/After Meal</b>			
$p_{\text{acid}}$ ratio	2.2	(1.7-2.9)	$P < 0.001$
$p_{\text{potassium}}$ ratio	5.2	(3.7-7.3)	$P < 0.001$
$p_{\text{calcium}}$ ratio	3.3	(2.3-4.6)	$P < 0.001$

TABLE X  
*Statistical Analysis: Duration of Active Plasma Level\**

Item	Value	Range (95 per cent confidence)	Significance for difference $\neq 0$
Fasting, difference between preparations			No significant difference
After meal, difference between preparations			No significant difference
Difference, after meal/fasting	1.1 hours (all preparations combined)	(0.7–1.5)	$P < 0.001$ (all preparations combined) $P < 0.01$ (for separate preparations)

\* Limit set at 0.05 u./ml.; result checked, whenever possible, also for 0.03 u./ml.

TABLE XI  
*Statistical Analysis: Penicillin in Urine, 0 to 4 Hours (u')*

Item	Value (geom. mean)	Range (95 per cent confidence)	Significance for ratio $\neq 1$
Fasting			
$u'_{\text{acid}}$	46600 u.		
$u'_{\text{salt}}$	78500 u.		
Ratio $u'_{\text{salt}}/u'_{\text{acid}}$	1.7	(1.3–2.2)	$P < 0.001$
Ratio $u'_{\text{potassium}}/u'_{\text{calcium}}$			Not significant
After Meal			
$u'$	47300 u., all preparations combined		No significant difference between preparations
Ratio: Fasting/After Meal			
$u'_{\text{acid}}$ ratio			Not significant
$u'_{\text{salt}}$ ratio	1.7	(1.3–2.0)	$P < 0.001$

TABLE XII  
*Statistical Analysis: Penicillin in Urine, 4 to 8 Hours (u'')*

Item	Value (geom. mean)	Range (95 per cent significance)	Significance for ratio $\neq 1$
Fasting			
$u''_{\text{acid}}$	3230 u.		
$u''_{\text{salt}}$	1670 u.		
Ratio $u''_{\text{acid}}/u''_{\text{salt}}$	1.9	(1.3–2.4)	$P < 0.01$
Ratio $u''_{\text{calcium}}/u''_{\text{potassium}}$			Not significant
After Meal			
$u''$	6900 u., all preparations combined		No significant difference between preparations
Ratio: After Meal/Fasting			
$u''_{\text{acid}}$ ratio	2.1	(1.4–3.1)	$P < 0.001$
$u''_{\text{salt}}$ ratio	4.1	(2.8–6.0)	$P < 0.001$

other published material,<sup>7</sup> while other available publications give no clear information on this point (e.g., different test subjects).

It can be subject to discussion which is to be preferred, a higher peak or a longer duration. In our opinion it is probably not of decisive importance if a peak for a short period amounts to one or to a few units/ml. of plasma. Repeated at adequate intervals, both will undoubtedly provide sufficient tissue concentrations for effective treatment of most penicillin sensitive infections.

A difference in duration, amounting to one hour or more, is likely to be of more practical importance, not least when only three or four daily doses are given, a regimen that is gaining popularity for reasons of simplicity.

Consequently, we do not feel that dosage of penicillin V on a fasting stomach should be preferred; in practice such a regimen is not very easy to follow strictly, and it gives active blood levels of a shorter duration than those obtained after a meal. "Regardless of meals" is a simple and quite acceptable principle, meaning in practice that most doses are not given on an empty stomach.

Another interesting question is whether one of the tested penicillin V preparations is more effective than any other.

First it may be stated that no differences whatever could be found between large and fine crystals of penicillin V acid.

The potassium salt gives a significantly higher peak than the acid, when given on a fasting stomach. This reflects the fact that the salt is readily dissolved and partly absorbed already in the stomach, whereas the acid is little soluble unless neutralized. Furthermore, it has been demonstrated recently in dogs<sup>10</sup> that absorption of penicillin V does occur through the stomach wall and not only in the intestines.

As the higher peak of the potassium salt is obtained only on a fasting stomach, the practical advantage of this peak seems to be reduced, if it is agreed that penicillin V preparations are best given after a meal.

Continuing the comparison of penicillin V acid and potassium salt, we found (fig. 2) a tendency for the salt to give a shorter duration, although the difference was not statistically proved. The 4 to 8 hour urine recovery of penicillin (fasting), however, was significantly lower for the salt than for the acid, suggesting that the differences in duration can be real, albeit small. In this connection we recall an experiment carried out earlier by Brante et al.<sup>13</sup>

In this study 10 healthy persons were given a subcutaneous injection of histamine (0.1 mg./10 Kg. of body weight) to stimulate formation of gastric acid. Thirty minutes later the persons, still fasting, got an oral dose of 200,000 units of potassium penicillin V. Plasma levels (1 and 3 hours) and urinary recovery (0 to 1 and 1 to 4 hours) were determined. The experiments were repeated on the same subjects but without histamine. After histamine the one hour plasma level (average) was 1.8 u./ml., without histamine 2.9 u./ml. Urinary recovery (0 to 4 hours) was one-third lower in the histamine series.

Similar experiments (fasting) with penicillin V acid (partly published<sup>2</sup>) did not show any major differences caused by the histamine stimulation, which demonstrates *in vivo* the better stability of penicillin V acid at a low pH.

It is thus conceivable that in patients with hyperacidity the penicillin V acid might provide a wider margin of safety than the potassium salt.

With such possible exceptions, the practical differences between penicillin V acid and its potassium salt are small and probably without importance for the average patient.

The calcium salt of penicillin V, as would be expected from its solubility, shows an uptake and an excretion between that of the acid and the potassium salt, being more closely related to the latter.

#### SUMMARY

Four types of penicillin V were given as rapidly disintegrating tablets to 12 healthy subjects: Penicillin V acid, large crystals (100 to 200  $\mu$ ); penicillin V acid, fine crystals (5 to 10  $\mu$ ); penicillin V potassium; penicillin V calcium.

Each preparation, 250,000 units, was given on a fasting stomach and one-half hour after a standardized meal. Plasma levels and urinary recovery of penicillin were determined in all eight series. The results were subjected to statistical analysis.

All preparations gave a lower peak but longer duration after a meal than on a fasting stomach. The longer duration makes dosage after meal preferable. No difference was found between large and fine crystals of penicillin V acid. The differences between penicillin V acid and potassium salt are noted, and the practical aspects are discussed. For the average patient the differences are probably without practical importance, but exceptions may exist. The calcium salt of penicillin V stands between the potassium salt and the acid with regard to absorption and excretion. There is fairly good correlation between plasma peak values and 0 to 4 hour urinary excretion of penicillin V, and also between duration in plasma and 4 to 8 hour urinary excretion.

#### ACKNOWLEDGMENT

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#### BIBLIOGRAPHY

1. BRANDL, E.; GIOVANNINI, M., AND MARGREITER, H.: Untersuchungen über das säurestabile, oral wirksame Phenoxymethylpenicillin (penicillin V), Wien. med. Wchnschr. 103:602, 1953.
2. SJÖBERG, S.-G.; BRANTE, G., AND RIGNÉR, K.-G.: Penicillin V, ett peroralt syrastabilt penicillin, Svenska Läkartidn. 52:83, Jan., 1955.
3. PUTNAM, L. E.; WRIGHT, W. W.; DE NUNZIO, A., AND WELCH, H.: Penicillin blood concentrations following oral administration of various dosage forms of penicillin V and comparison with penicillin G. In: Antibiotics Annual 1955-1956, New York, Medical Encyclopedia, Inc., 1956, p. 483.
4. KAIPAINEN, W. J., AND HÄRKÖNEN, P.: Serum concentrations after oral administration of phenoxymethylpenicillin acid and phenoxymethylpenicillin potassium, Scandinav. J. Clin. & Lab. Investigation 8:18, 1956.
5. COLQUHOUN, J.; SCORER, E. C.; SANDLER, G., AND WILSON, G. M.: Absorption of free acid, potassium, and benzathine phenoxymethylpenicillin after oral administration, Brit. M. J. 1:1451, June, 1957.
6. JUNCHER, H., AND RAASCHOU, F.: The solubility of oral preparations of penicillin V, AM & CT 4:497, Sept., 1957.
7. PECK, F. B., JR., AND GRIFFITH, R. S.: Comparative clinical laboratory studies of potassium penicillin V with acid penicillin V. In: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 1004.
8. WRIGHT, W. W., AND WELCH, H.: Oral potassium penicillin V. Serum concentrations and urinary excretion, AM & CT 5:139, Feb., 1958.
9. GIOVANNINI, M., AND KRAUSHAAR, A.: Die Ausscheidung verschiedener Penicilline mit dem Harn, Arzn.-Forsch. 8:86, 1958.
10. LEE, C.-C.; FROMAN, R. O.; ANDERSON, R. C., AND CHEN, K. K.: Gastric and intestinal absorption of potassium penicillin V and the free acid, Antib. & Chemo. 8:354, July, 1958.
11. MÖLLER, O., AND ÅKERRÉN, B.: Penicillin peroralt, Svenska Läkartidn. 55:2277, Aug., 1958.
12. BRANTE, G.; SJÖBERG, S.-G., AND AHLINDER, S.: Peroral behandling med DBED-penicillin V i vattensuspension. Svenska Läkartidn. 55:2534, Sept., 1958.
13. BRANTE, G.; SJÖBERG, S.-G., AND RIGNÉR, K.-G.: Unpublished data.

# Comparative Investigations with Acid Penicillin V and a Water-soluble Penicillin V Salt under Different Conditions

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Juncher and Raaschou<sup>1,2</sup> and Peck and Griffith<sup>3</sup> compared water-soluble salts of acid penicillin V and free acid after oral administration and found that potassium penicillin V produces higher blood levels than acid penicillin V. These results appeared to be contradictory to those obtained by earlier workers,<sup>4,7</sup> and it was necessary to find out whether in this case new properties of the substance investigated were concerned or whether the results obtained might, in some respect, depend on the experimental conditions prevailing in each special case. The present paper deals with such comparative investigations with the aim of establishing definite rules concerning the dependence of the blood level on the administered penicillin V substances.

The first problem to be solved was that of dosage, especially since the aforementioned workers differ from us in regard to this matter.

The second difficulty to be overcome in explaining the discrepancies was in relation to the conditions of resorption, which are of particular importance in view of the varying degrees of acid resistance of the substances investigated. It was therefore necessary to carry out, in addition to experiments performed with patients whose stomachs were empty, which are of theoretical interest only, comparative investigations adapted to what actually occurs in practice, i.e., with persons with at least a partly filled stomach.

We conducted our tests with persons in good health, who were examined both when their stomachs were empty and also after breakfast (i.e., after having eaten coffee with milk and sugar and two slices of toast, about 100 Gm., and butter). The substances to be examined were administered in soft capsules, without the addition of any vehicle substances, with about 100 ml. of water. Blood (about 3 ml.) was taken from the cubital vein, and the serum obtained after centrifugation was studied in a serial dilution test with respect to its content of active penicillin by means of the test strain *Staphylococcus aureus* SG-511. Potassium penicillin V served as a standard for comparison. At least 10 experiments were carried out for each point of the blood level curves.

## RESULTS

Figure 1 shows penicillin blood levels after the oral administration of 60 mg. acid penicillin V or potassium penicillin V to test subjects with an empty stomach. This dosage was used in our studies because the blood levels attained with it had for a long time been considered adequate, at least in Europe, and because most of the earlier publications are based on this dosage. Certain differences are already indicated by these blood level curves, a phenomenon that manifests itself even more clearly in the case of high dosages, namely, by a more rapid resorption and a correspondingly higher blood level in the course of the first hour after potassium penicillin V, which is easily soluble in water, has been administered. It is likewise made clear that this substance, being less acid-resistant than the free acid, is quickly destroyed, so that the blood level is soon reduced.

FIG. 1. Shown is the penicillin blood level after the peroral administration of 60 mg. penicillin V acid (—) or potassium penicillin V (----) on an empty stomach.

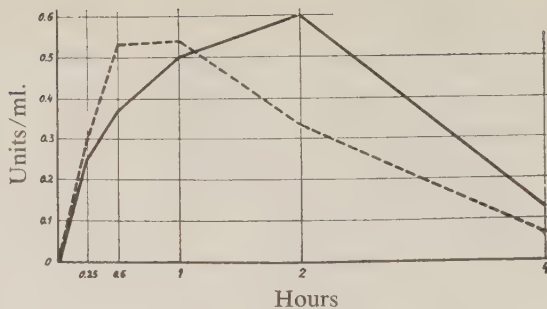


Figure 2 shows levels attained after a double dosage (120 mg.) was administered. In this case the blood level of potassium penicillin V will exceed that of acid penicillin V only during a period of from about 30 to 40 minutes after the preparation has been administered, whereas at all later periods penicillin V acid will have higher values. Also, the highest possible peak will, in the case of this dosage, be attained by acid penicillin V, although the difference will not be great.

The work carried out by the afore-mentioned authors relates mainly to the highest dosage of 240 or 250 mg. It remains for the physician attending the patient to decide whether this extremely high dosage is necessary. In our case, this problem was investigated by the Department of Antibiotic Research of the First University Clinic of Vienna. Another paper will deal with the work carried out in this connection. It was our task merely to solve the problem of blood level, and for this purpose we carried out experiments with 240 mg. potassium penicillin V and acid penicillin V. The results obtained are shown in figure 3.

The more rapid resorption of water-soluble potassium penicillin V, which leads to a maximum value after only 30 minutes if such high dosages are administered, is clearly shown. We were, however, not able, in the course of this series of experiments, to reproduce the results obtained by Peck and Griffith,<sup>3</sup> who attained maximum values of 7 units/ml. Looking back on the numerous experiments we carried out with these high dosages, we must reach the conclusion that in the case of a small number of test subjects, it is possible to attain such maximum values, provided that resorption conditions are particularly favorable as a result of high pH values in the stomach. If, however, the number of experiments is multiplied, these values of the

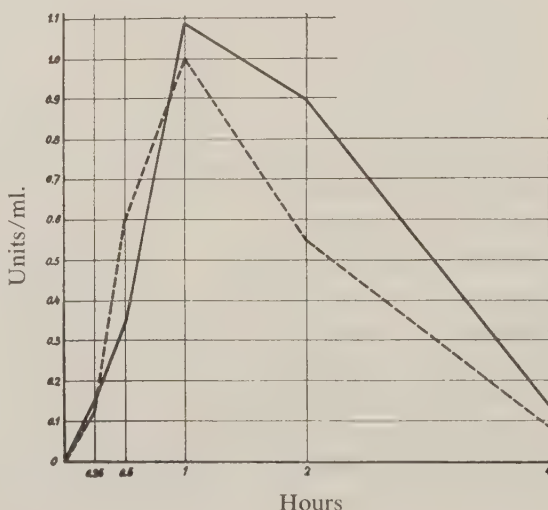


FIG. 2. Shown is the penicillin blood level after the peroral administration of 120 mg. penicillin V acid (—) or potassium penicillin V (----) on an empty stomach.

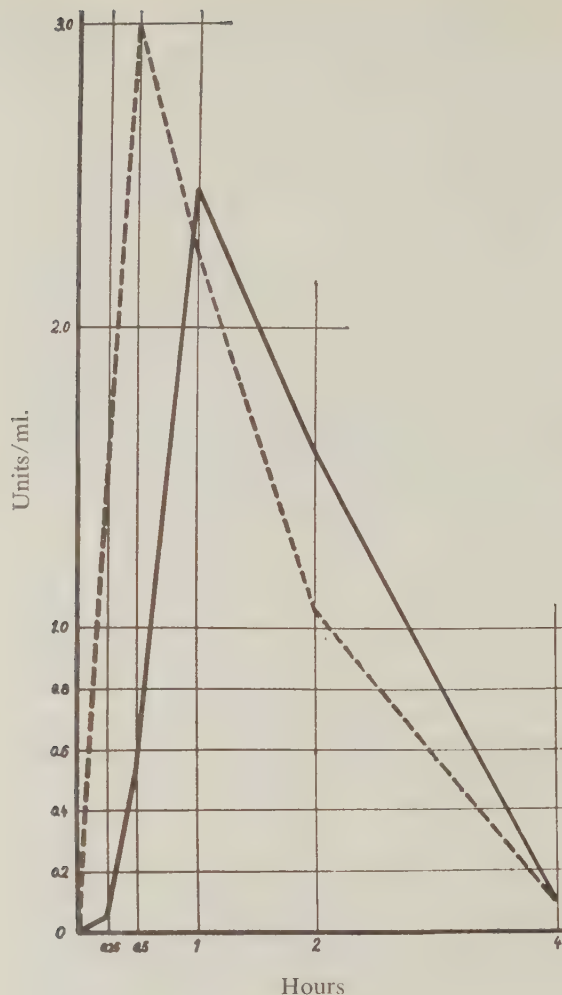


FIG. 3. Illustrated is the penicillin blood level after the peroral administration of 240 mg. penicillin V acid (—) or potassium penicillin V (----) on an empty stomach.

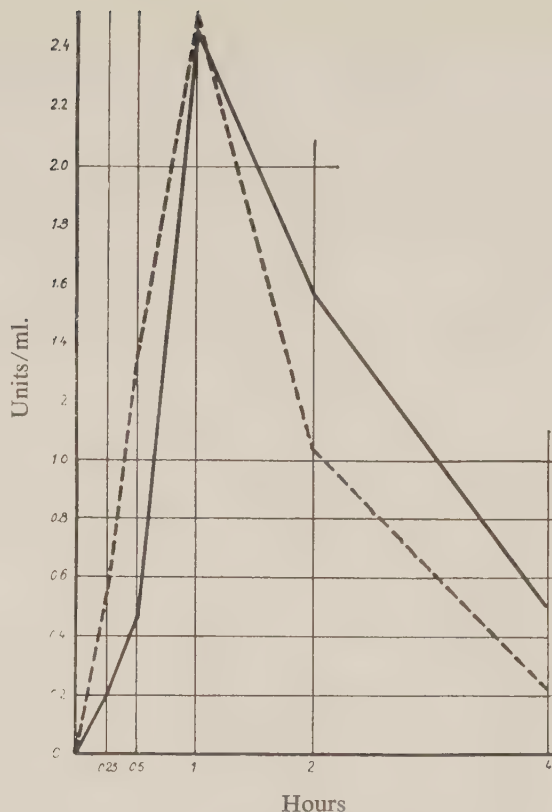
initial blood level are reduced considerably, since in most persons the stomach  $pH$  is of such an order of magnitude that potassium penicillin V is destroyed to a considerable extent.<sup>8</sup>

Figure 3 shows that acid penicillin V attains higher later values than potassium penicillin V also in this case, which may readily be explained by the greater acid resistance of this substance.

We consider it of the utmost importance that in the course of such investigations, which are intended to assist physicians in deciding on the correct treatment in each individual case, conditions such as actually exist in practice be duly taken into account. It will be found practically impossible to administer penicillin on an entirely empty stomach. We therefore organized a series of experiments based on such conditions. If potassium penicillin V and acid penicillin V are administered in standard dosages of 60 and 120 mg., respectively, acid penicillin V is naturally utilized to a greater extent after meals than salt, even if these drugs are administered on an empty stomach, as is clearly shown in our illustrations. We must therefore be satisfied with describing conditions prevailing if extremely high dosages are administered.

Whereas the curves obtained by Peck and Griffith<sup>3</sup> demonstrate the surprising fact that acid penicillin V is resorbed more quickly after meals, in contradiction to what was found as a result of experiments carried out by the authors with test sub-

FIG. 4. Illustrated is the penicillin blood level after the peroral administration of 240 mg. penicillin V acid (—) or potassium penicillin V (----) to test persons after a trial breakfast.



jects whose stomachs were empty, and that potassium penicillin V has higher values later, the experimental results obtained by us agree with those carried out on persons with an empty stomach, i.e., potassium penicillin V is absorbed more rapidly and also its blood level values decrease more rapidly than in the case of acid penicillin V. However, the essential difference between the two substances under investigation consists in the fact that the high initial peak of potassium penicillin V in the case of empty-stomach medication decreases by about 20 per cent in tests carried out by us and by nearly 50 per cent in the case of those carried out by Peck and Griffith, whereas the peak values of acid penicillin V were not influenced by meals either in experiments carried out by us or in those performed by the afore-mentioned authors. The continuation of the curve after meals distinctly shows the effect produced by the higher degree of acid resistance found in acid penicillin V, and it is important to note that at the end of the curve, values are still twice as high as in the case of the potassium salt. In the course of more recent investigations, which were carried out in connection with other problems, we were still able to observe therapeutic blood levels (more than 0.1 unit/ml.) six hours after acid penicillin V had been administered (fig. 4).

In summarizing the results, it may be said with respect to these experiments that in the case of patients with an empty stomach, potassium penicillin V causes high initial levels, which may be explained by the fact that it is present in the form of a solution in the empty stomach, and that it immediately passes through the pylorus and is quickly resorbed in the duodenum. The portion remaining in the stomach is destroyed by the acid gastric juice, which now takes effect, so that in the course of further development only a smaller quantity, i.e., the still undestroyed portion, can be resorbed. This also explains the higher initial value in the case of a 240 mg.

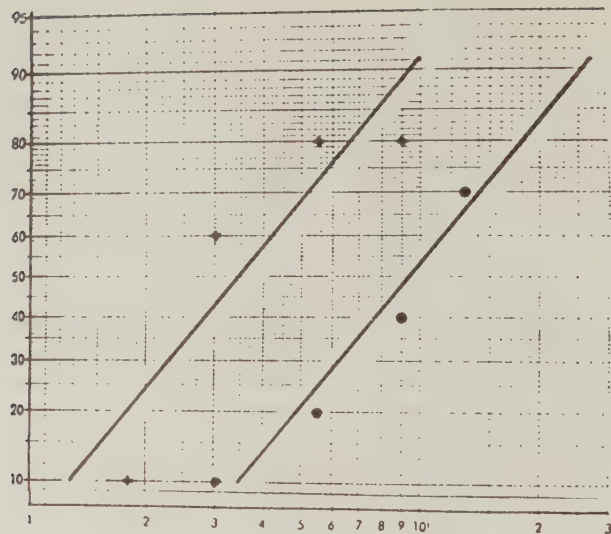


FIG. 5. The dose effect times of penicillin V acid (left) and potassium penicillin V (right), each point is 10 mice, against infection with *Streptococcus hemolyticus* Aronson.

dosage, for the higher the dosage, the greater will probably be the undestroyed portion of the salt that immediately passes through the stomach. The opposite is found in the case of acid penicillin V. Because of the low degree of water-solubility of this substance, immediate resorption in the duodenum is not possible, and a gradual increase of blood level values may be expected with normal dosages. However, since acid penicillin V is almost not destroyed by gastric juices at all because it is almost fully acid-resistant, it is possible for resorption to take place during the entire period in the duodenum. These differences are especially noticeable if the preparations are administered after meals. Since the stomach is full, the pylorus passage narrows and only small portions of the contents of the stomach can pass into the duodenum, so that it is not possible for larger quantities of potassium penicillin V to be resorbed. The portion remaining in the stomach is destroyed, so that the absolute amounts of resorption are also reduced. Acid penicillin V, on the other hand, is not influenced at all by these processes because of its acid resistance.

Apart from blood level determinations, we obtained other more direct evidence of a possibility of clinical superiority of penicillin V acid over penicillin V salt.

When we determined the oral protective doses of both drugs required to protect 50 per cent of mice, infected with an otherwise lethal dose of *Streptococcus hemolyticus* Aronson, the following preliminary results were obtained (fig. 5). In the statistical evaluation it is shown that in the slope function there is no difference [penicillin V acid, 2.17 (1.24 to 3.80), and potassium penicillin V, 2.16 (1.17 to 4.03)], which means that the mechanism of action is qualitatively the same. Quantitatively, on the other hand, there is a significant difference. The potency ratio of the  $ED_{50}$  is 2.75 and it shows that penicillin V acid is significantly more active than potassium penicillin V, 9.50 units/Gm. of body weight is required to afford this protection, whereas the same results may be obtained with 3.45 units of the free acid. For confidence limits of 19/20 the relative activity of the penicillin V acid lies between 1.61 and 4.78 times that of potassium penicillin V.

#### CONCLUSION

Penicillin blood levels, after oral administration on an empty stomach, are likely to increase more rapidly in the case of potassium penicillin V than in the case of

acid penicillin V, and initial values are higher with high dosages (240 mg./dose). On the other hand, later values are higher with acid penicillin V than with the salt. These phenomena are explained by the conditions of solubility and by the corresponding amount of resorption within the time period. If the stomach is not quite empty (e.g., after breakfast), the high initial values of potassium penicillin V decrease considerably, whereas blood levels remain nearly unchanged after acid penicillin V has been administered. These facts depend on the acid stability of the substances. Conclusions drawn from these investigations as applied to clinical practice are discussed. In experiments with infected mice it was shown that, in the median protective doses, the acid penicillin V is statistically significantly more active than the potassium penicillin V.

#### BIBLIOGRAPHY

1. JUNCHER, H., AND RAASCHOU, F.: Penicillinkonzentrationen im Plasma nach oraler Anwendung von Penicillin V, *Ugesk. f. læger* 117:1131, 1955.
2. JUNCHER, H., AND RAASCHOU, F.: Resorption und Löslichkeit oraler Penicillin-V Praeparate, *Ugesk. f. læger* 119:607, 1957.
3. PECK, F. B., JR., AND GRIFFITH, R. S.: Comparative clinical laboratory studies of potassium penicillin V with acid penicillin V. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 1004.
4. BRANDL, E.; GIOVANNINI, M., AND MARGREITER, H.: Untersuchungen über das sauerestabile, oral wirksame Phenoxymethyl Penicillin (Penicillin V), *Wien. med. Wchnschr.* 103:602, 1953.
5. SCHINDELMAISSER, F.: Erfahrungen eines praktischen Arztes mit "Ospen," Penicillin V, einem säurestabilen Oralpenicillin, *Wien. med. Wchnschr.* 104:369, 1954.
6. ELIAS, W. F., AND MERRION, H. J.: Some observations on penicillin V. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, p. 510.
7. RUPPERT, A., AND ROTTER-POOL, P.: Erste Erfahrungen mit Penicillin-V-Säure in der operativen Gynaekologie, *Deutsches med. J.* 6:15, 1955.
8. BRUNNER, R., AND MARGREITER, H.: Löslichkeitsprobleme der Basensalze der Penicilline, *Monatschr. f. Chemie* 86:958, 1955.

RICHARD BRUNNER

We heard Berlin and Brante report that the penicillin blood levels after administration of any penicillin V preparations show the peak occurring later and being maintained for a longer time on therapeutic doses given after meals than on an empty stomach. Therefore, the penicillin V medication after meals is superior to the penicillin V medication on an empty stomach. Thus from these blood level curves and resorption data (urinary excretion), the areas in the case of potassium penicillin V decrease much more than in the case of free penicillin V acid, if change is made from medication on an empty stomach to medication after meals.

Therefore, according to Berlin and Brante, the excretion of given potassium penicillin V decreases from 34 to 23 per cent, while in the case of the free acid the excretion increases, but not significantly, from 22 to 26 per cent. We, and many other people, obtained similar findings. This shows that the potassium penicillin V will be partially destroyed if it stays in the stomach for a longer period, which is not true for penicillin V acid. Therefore, the penicillin V acid is in our opinion superior to the salt, and administration orally after meals is the best. This superiority is remarkable with small doses, but the difference can also be seen with high doses. In penicillin oral therapy it is essential to have continuous and safe blood levels, such as those obtained with the free acid. The higher blood levels obtained in some cases with the potassium salt are in our opinion of no therapeutic importance.

Furthermore, this, the superiority of the free penicillin V acid, is shown by the animal experiments given in the preliminary report by Kraushaar and Giovannini.

## Discussion of the Papers by Kraushaar and Giovannini

KARL SPITZY

We have numerous reports on blood level studies after single doses of penicillin V. Penicillin V was given both on a fasting stomach and after a meal. The oral therapy should, however, be repeated every four to six hours. In addition, the gastric acidity is essential for the difference between penicillin V and its salts.

For this reason, we have performed blood level studies with 250 mg. of penicillin V acid and potassium salt, respectively, given in repeated doses every six hours. Also, the gastric acidity was checked. In 7 cases with acidity values higher than 20 (which corresponds to a  $pH$  lower than 1.8) a higher blood level from the potassium salt was obtained only in the first dose and during the first hours. After six hours, the level from the salt is lower than that of the acid. After two or three doses, the levels obtained from the acid were higher, both at one hour and six hours.

In repeated dosage, with a normal diet and in cases with normal and even more in cases with excessive gastric acidity, the therapy with penicillin V acid is superior to that with the penicillin V salt. This fact was already stressed by our group five years ago.

The animal experiments conducted by Kraushaar and Giovannini seem to give sufficient evidence to finish the discussion of this matter.

It is not necessary to await the clinical proof, I think, of the superiority of the penicillin V acid and thus risking clinical failures by failing to use it.

# Blood Levels after Oral Administration of Penicillin V Acid or Penicillin V Salt in Relation to Gastric Acidity

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At the very beginning of our studies on phenoxymethyl penicillin V, in 1953, we used not only free acid but also the salts of phenoxymethyl penicillin.<sup>1</sup> We found that the acid stability of the free acid of penicillin V in vitro was far superior to the alkaline salt.<sup>2,3</sup> We also found that with a dosage of 60 mg., orally administered penicillin V free acid was better utilized and achieved higher blood levels than potassium penicillin V.

More recent observations indicate that with a 250 mg. dosage the initial level was higher with potassium penicillin V.<sup>4,5</sup> Studies on healthy subjects revealed that such a difference exists only if higher doses are given on an empty stomach.<sup>6</sup> However, all investigators agreed that penicillin V free acid produces longer lasting high levels than potassium penicillin V.

It was therefore concluded that utilization of penicillin V salt is better or at least equal to the acid if given on an empty stomach. If taken with or after a meal, the V salt is not so well utilized. The cause for this is based on the greater sensitivity of the potassium salt to gastric acidity. We believed therefore it would be interesting to study patients whose gastric juice pH was low.

The blood levels of 18 patients after a single dose of a 250 mg. penicillin V acid and 250 mg. potassium penicillin V were checked. Eighteen patients received penicillin V preparations.\* No significant difference was found. However, the Austrian preparation seemed superior and made the difference between penicillin V free acid and penicillin V salt more conspicuous. In hospitalized patients who did not have gastroenteritis and who did not have any hepatic, renal, or cardiac diseases, a probe was put into the stomach, and the gastric juice was analyzed and expressed in *N*/10 hydrochloric acid in free and bound acid. If the acidity of the specimen was 20 or higher, the patient received a 250 mg. dose, and after 0, ½, 1, 2, 4, and 6 hours blood was taken from the cubitalis vena and the serum blood levels determined after the serial dilution method of Dornbush and Pelcak against *Staphylococcus aureus* (tables I and II).

The Austrian preparation, which seemed to achieve more favorable results in our experiments, revealed only in the first half hour higher levels for penicillin V salt in cases with higher gastric acidity. The difference was 1½/test tube. After an hour both values were the same, after two hours the level for penicillin V salt was half a test tube lower, and in the fourth hour almost two test tubes lower than that for penicillin V acid. In the sixth hour the levels for potassium penicillin V were below the curative level.

In order to observe the serum levels of patients during actual treatment, the gastric acidity was determined continuously in 7 patients. The amount of gastric acidity was registered before administration of penicillin V salt capsules every six hours and compared with the value for penicillin V acid. Every sixth and seventh hour blood samples were taken and the serum level determined (table III).

\* Eleven patients received a preparation from Biochemie and seven received the product produced by Eli Lilly & Co.

TABLE I

*Blood Levels after the Peroral Administration of 250 mg. Penicillin V Acid and Salt (Biochemie, Austria)*

Pt.	Penicillin V acid						Penicillin V salt				
	Gastric acid value (N/10)	½ hr.	1 hr.	2 hr.	4 hr.	6 hr.	Gastric acid value (N/10)	½ hr.	1 hr.	2 hr.	4 hr.
Sp	40/65	—	—	1.60	0.49	0.195	35/70	—	1.50	1.00	—
Pr	50/72	—	3.38	0.83	0.365	0.058	60/90	—	1.88	0.44	0.44
So	53/75	—	5.07	1.00	0.058	0.058	70/95	11.39	6.34	0.83	0.13
Fe	68/87	—	1.00	2.81	0.29	0.039	65/80	3.38	1.00	0.44	0.072
Fr	48/76	5.06	3.38	0.83	0.039	0	35/70	—	2.25	1.88	0.049
Op	40/75	0.365	1.00	2.25	1.00	0.44	35/60	5.07	3.38	1.50	0.29
Ob	40/60	5.07	3.38	0.66	0.195	—	45/60	7.60	2.25	0.365	0.039
Mö	40/50	3.38	3.38	0.83	0.242	—	50/75	5.07	2.25	0.83	—
Ge	45/53	3.38	3.38	1.50	0.039	—	62/80	2.25	2.25	0.66	1.195
Ba	46/80	0.29	1.00	0.66	0.87	—	60/80	2.25	2.25	2.25	0.049
Pr	65/85	1.50	2.81	0.66	0.039	—	64/88	5.07	2.25	1.00	0.087
Wa		3.38	3.38	5.06	0.44	—					
Av.		2.72	2.78	1.24	0.258			5.28	2.51	1.018	0.123

It could be shown that the levels with potassium penicillin V were only greater than those with penicillin V acid; after 12 hours and at later intervals, the penicillin V acid levels are superior. The levels six hours after delivering of the agent were throughout our experiments ineffective. This might also account for the production of higher levels in the seventh and thirteenth hour with penicillin V acid.

On the basis of our studies we recommended five years ago that phenoxymethyl penicillin free acid be used for oral penicillin therapy. We were well aware at this time the potassium penicillin V was more soluble and its active resorption superior. For oral therapy, potassium penicillin seemed less suitable, since the penicillin free acid was more stable in the acid medium of the gastric juice. These properties are more manifest with the use of smaller doses (60 and 120 mg. for single dose) where the advantageous resorption of potassium penicillin V does not come into consideration.

With a dosage greater than 120 mg. given on the empty stomach the initial level with potassium penicillin V is higher as a result of the more rapid resorption. However, the terminal levels are lower than with penicillin V acid. If taken after a meal

TABLE II

*Blood Levels after the Peroral Administration of 250 mg. of Penicillin V Acid and Salt (Eli Lilly & Co.)*

Pt.	Penicillin V acid					Penicillin V salt				
	Gastric acid value (N/10)	½ hr.	1 hr.	2 hr.	4 hr.	Gastric acid value (N/10)	½ hr.	1 hr.	2 hr.	4 hr.
Pf	35/58	0.162	1.50	1.50	0.195	30/62	2.81	5.07	1.00	0.13
Hö	58/85	0.049	1.50	1.50	0.29	40/72	7.60	5.07	1.00	0.087
Se	32/48	0.66	1.00	1.00	0.108					
Kr						44/90	2.81	1.25	0.44	0.195
Ne						20/58	0.13	4.27	2.25	0.44
Ri	25/40	0.195	0.195	0.44	0.29	30/56	1.25	1.88	2.81	—
Rie	40/62	0.242	2.25	3.38	1.50					
Wa	50/75	1.50	5.07	0.66	0.039					
Fr						28/70	1.50	2.25	1.00	0.087
Hu						20/40	0.058	0.13	2.25	0.29
Av.		0.47	1.92	1.41	0.40		2.31	2.84	1.53	0.21

TABLE III

*Penicillin Blood Levels after Administration of 250 mg. of Penicillin V Acid and Salt Every Sixth or Seventh Hour*

Penicillin V acid								Penicillin V salt							
Gastric acid value		First dose		Second dose		Third dose		Gastric acid value		First dose		Second dose		Third dose	
Pt.	(N/10)	0	1 hr.	6 hr.	7 hr.	12 hr.	13 hr.	(N/10)	0	1 hr.	6 hr.	7 hr.	12 hr.	13 hr.	
We	80/120	0	0.29	0.66	0.13	0.13	0.162	60/90	0	1.88	0.058	0.44	0.058	1.50	
Kl	23/70	0	1.88	0.039	1.88	0.039	1.00	40/90	0	0.039	0.039	0.66	0.039	0.66	
Sch	40/100	0	0.66	0.108	—	2.25	—	20/40	0	2.81	0.039	2.24	0.108	3.38	
Kr	44/90	0	2.23	0.058	2.25	0.039	2.25	50/110	0	2.25	0.030	1.50	0.039	0.66	
Wa	80/120	0	2.25	0.13	7.60	0.29	5.07	30/60	0	2.25	0	0.55	0.058	1.50	
Ha	60/110	0	2.25	0.44	0.55	0.44	3.38	28/90	0	5.07	—	—	—	—	
La	40/80	0	1.88	0.139	2.25	0.75	2.25	36/82	0	2.25	0.039	1.50	0	1.00	
Av.		0	1.59	0.24	2.44	0.19	2.37		0	2.38	0.035	1.00	0.08	1.34	

or especially with high free acid in the stomach, the initial higher level cannot be obtained. Most noticeable are these differences if the serum levels are controlled during the course of a penicillin treatment. If penicillin is given orally at four to six hour intervals, the level of the antibiotic in the serum reaches higher values with penicillin V acid than with potassium penicillin V (table III). It is therefore apparent that potassium penicillin V is by no means an advantage over penicillin V acid.

From our experiments and with the preparations we used it became clear that penicillin V acid, considering all important criteria of an antibiotic, proved superior to potassium penicillin V.

#### SUMMARY

In the 25 patients whose gastric juice had more than 20 free acid ( $pH$  1.5) capsules of 250 mg. penicillin V acid and potassium penicillin V were dispensed orally in single doses or continued every six hours. The serum levels were checked. After a single dose higher 30 minutes' values were obtained with potassium penicillin V than with penicillin V acid; after one hour the values were equal, after two, four, and six hours they were higher with penicillin acid. If the antibiotic was dispensed every six hours the initial level after an hour was slightly higher with the potassium penicillin V; after 12 and 18 hours, respectively, the penicillin V acid was definitely superior.

#### BIBLIOGRAPHY

1. SPITZY, K. H.: *Wien klin. Wchnschr.* 65:936, 1953.
2. BRANDL, E.; GIOVANNINI, M., AND MARGREITER, H.: Untersuchungen über das säuerstabile oral wirksame Phenoxymethyl Penicillin (Penicillin V), *Wien med. Wchnschr.* 103:602, Aug. 8, 1953.
3. SPITZY, K. H.: Die perorale Penicillintherapie. In: Gsell, O., Ed.: *Antibiotica et Chemotherapie*, Vol. 2, Karger, Basel, 1955, p. 134.
4. JUNCHER, H., AND RAASCHOU, F.: *Ugesk. f. laeger.* 119:607, 1955.
5. PECK, F. B., JR., AND GRIFFITH, R. S.: Comparative clinical laboratory studies of potassium penicillin V with acid penicillin V. In: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 1004.
6. KRAUSHAAR, A., AND GIOVANNINI, M.: Comparative investigations with penicillin V acid and a water-soluble penicillin V salt under different conditions. In: *Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 158.

# Investigations of a Depot Effect Produced by Phenoxymethyl Penicillin (Acid Penicillin V)

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After the introduction of penicillin, injection therapy of this antibiotic was carried out with water-soluble alkali salts. Because of its rapid absorption and also its rapid excretion, injections were necessary at intervals of four hours. Many attempts have been made to reduce the number of injections. In this connection, the production of procaine penicillin G represents a decisive step because this product, in addition to its supplementary anesthetic effect, makes the injections painless, and because of its low solubility, it has a depot effect, which makes possible fewer injections. The first investigations were done by Herrell and Nichols,<sup>1</sup> who found that a therapeutically sufficient blood level results after intramuscular administration of 300,000 units of procaine penicillin G in oil over 24 hours. More exact investigations have been carried out by another group,<sup>2</sup> who studied procaine penicillin G in different forms of administration (in peanut oil, in oil with aluminum stearate, and in aqueous suspension). These authors discovered that the suspension in oil with aluminum monostearate has the best depot effect, while procaine penicillin G in aqueous suspension falls below the therapeutic limit sooner but shows high initial blood levels. Since these reports, procaine penicillin G has been investigated by many authors, but these first results are still important for its therapeutic application. In spite of the introduction of a great number of additional penicillin G salts of low solubility, this product held first place as a 12 to 24 hour depot preparation until now.

Contrasted with these advantages of this form of therapy, we find some disadvantages, which we must point out. First, the frequent appearance of allergic symptoms caused by the procaine part of the molecule is important.<sup>3-7</sup> By applying other salts, attempts have been made to prevent this problem,<sup>\*9-15</sup> but until now no satisfactory solution has been found. A few years ago studies were made on an almost insoluble salt of penicillin V—DBED penicillin V<sup>†</sup> for parenteral administration.<sup>21-24</sup> The results of these tests showed that it is possible to get a depot effect lasting almost three days with DBED penicillin V.

Elimination of difficulties seemed to be possible with use of a penicillin preparation, which has no part in the molecule producing any side reactions. Under these circumstances, phenoxymethyl penicillin (acid penicillin V) has a great advantage having as cationic part the hydrogen molecule ion only. Since the molecule of benzylpenicillin (penicillin G) is not constant, it is not suitable. Although phenoxymethyl penicillin made possible the reliable oral application of penicillin because of its quality of acid stability, and therefore this form of administration has been most used until now, study of this interesting substance with regard to the problem of absorption and urinary excretion in the parental form of administration is surely justified from a scientific point of view.

## STUDIES

*Investigations on Rabbits.* METHOD. Rabbits with an average weight of 3.5 Kg. were not fed for 12 hours before beginning the tests and also received no liquid or

\* R. S. Hanslick, U. S. Patent 2,742,465 (1956).

† Austrian Patent No. 191546 (1954) by R. Brunner, H. Margreiter, and K. Riedl (Biochemie Ges.m.b.H., Kundl/Tyrol, Austria).

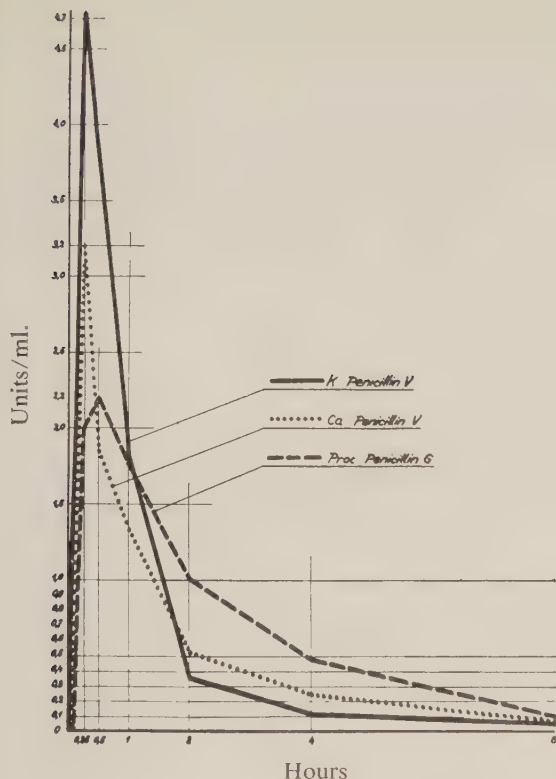


FIG. 1. Presented is a comparison of investigations of potassium penicillin V, calcium penicillin V, and procaine penicillin G, 10,000 units/Kg. administered intramuscularly to rabbits.

solid feeding during the duration of the test. The test substance was injected in one of the hind legs. Blood was taken from the ear vein in the intervals indicated in the figures. In order to ascertain inhibition of blood, a control experiment was carried out before each test. One tenth ml. of pure blood, taken by means of a micropipette, was tested in bisecting ranges and compared with specific standard penicillin against *Staphylococcus aureus* SG 511. Incubation was at a temperature of 37 C. for 14 hours. Coagulation of the serum was the criterion of overgrowth. For each substance to be tested at least 10 rabbits were used, and the mean value of these results is shown in the figures.

The first investigations were carried out at a low dosage with high-soluble and low-soluble salts of acid penicillin V, namely, with the potassium salt and the calcium salt; comparisons were made with procaine penicillin G. As shown in figure 1, extremely high blood levels resulted with the potassium salt of acid penicillin V, which reached the basic therapeutic value after about four hours. The top of the calcium penicillin V curve is essentially lower than and its descent is parallel to potassium penicillin V. The therapeutic blood level, however, is maintained some time longer. In comparison, procaine penicillin G, at the same dosage, shows a type of depot preparation with a relatively low initial blood level and a long end curve.

The preparations employed had the following water-solubility: potassium penicillin V, almost completely soluble; calcium penicillin V, 1.1 per cent; procaine penicillin G, 0.5 per cent. The resorption depends clearly on the water-solubility of the preparations, as has been shown in the urinary excretion experiments of penicillin salts with varying solubility.<sup>16</sup>

Theoretically, the experiments with potassium penicillin V leave open the possi-

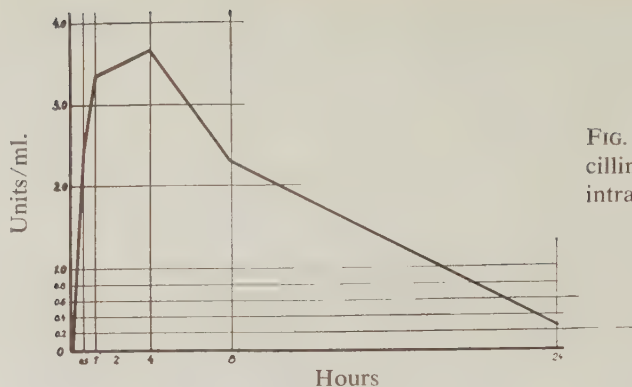


FIG. 2. The blood level for procaine penicillin G, 80,000 units/Kg. administered intramuscularly to rabbits, is shown.

bility that injected phenoxymethyl penicillin is quickly changed into a salt with the cations of the tissue and that it is absorbed, so that similar curves result as is shown in figure 1 with regard to penicillin V. It is surprising that this does not happen, as may be seen in figure 3.

Since the employed dosage of 10,000 units/Kg. does not clearly show an eventual depot effect, in the experiments described until now, in later experiments dosages of 80,000 units/Kg. were used. We subscribe to the view of Bauer et al.<sup>17</sup> that in testing a depot effect it is important to have identical proportions in man and animal, i.e., to inject the same dosage in the animal as is normally necessary for man. With regard to a later possible reduction in the true proportions with penicillin preparations of determined solubility, we refer once more to the publication mentioned.<sup>17</sup>

In order to determine the conditions of our experiments, we experimented with procaine penicillin G in aqueous suspension in the usual form (Hypropen) containing 300,000 units/ml. As is previously explained, we used 80,000 units/Kg. for a more exact dosage. The results of this investigation on 20 animals are described in figure 2. It clearly shows the depot effect of procaine penicillin G.

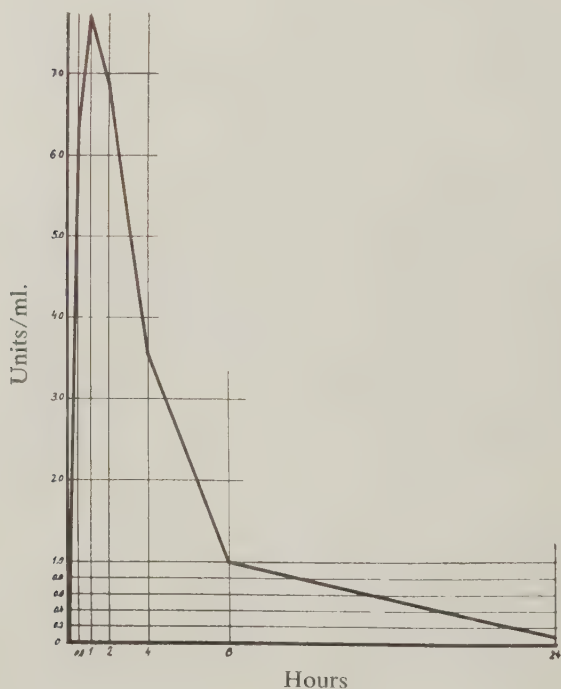
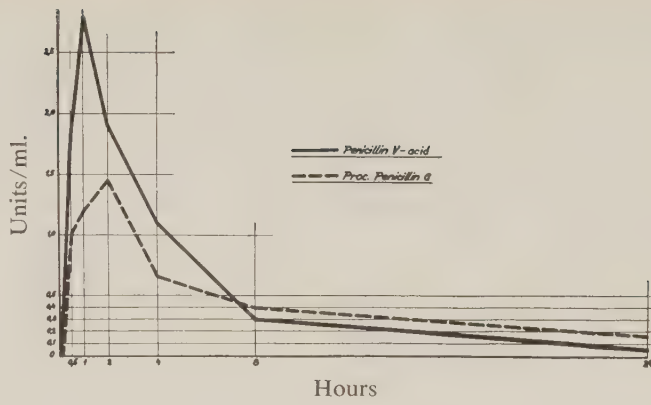


FIG. 3. The blood level is given for penicillin V acid, 80,000 units/Kg. administered intramuscularly to rabbits.

FIG. 4. The blood level for penicillin V acid and procaine penicillin G, 300,000 units administered intramuscularly in man, is shown.



Corresponding experiments with phenoxymethyl penicillin were carried out in exactly the same way to determine the quantity to use. Figure 3 shows the results of these experiments.

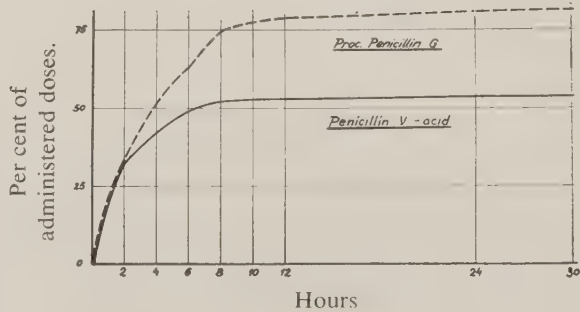
As is evident from experiments with rabbits, it is possible to maintain blood levels for a longer time with phenoxymethyl penicillin, with curves closely resembling those of procaine penicillin G. As the problem of absorption in relation to body weight is very important with antibiotics, corresponding experiments have been made on man.

*Experiments on Man. METHOD.* For these experiments, healthy persons, who continued their usual work during the duration of the experiments, were studied. Injections of the preparations were given deeply intramuscularly in a dosage of 300,000 units in the exterior part of the gluteus maximus. Three ml. of blood was taken from the cubital vein. The blood was centrifuged (3000 turns/minute) and the serum tested. Tests were made with *Staph. aureus* SG 511 (factor 2/3). Urinary excretion experiments were carried out by means of plate tests with *Staph. aureus* SG 511.<sup>16</sup>

In figure 4 the comparative studies between procaine penicillin G and acid penicillin V are depicted. It may be seen that acid penicillin V has a positive depot effect. The therapeutically necessary blood level of 0.1 units/ml. is maintained about 20 hours. Oddly, an essentially higher initial blood level is reached with acid penicillin V than with procaine penicillin G. The cross point of both substances is at nearly 7 hours.

In this regard the urinary excretion studies have been interesting; up to 12 hours, the urinary excretion was measured every 2 hours, and later, after 24 and 30 hours (fig. 5). As previously shown, about 80 per cent of the administered quantity of procaine penicillin G was excreted, whereas only 54 per cent of acid penicillin V was excreted.

FIG. 5. The urinary excretion of procaine penicillin G and penicillin V acid after an administration of 300,000 units intramuscularly in man is given.



Because of the high solubility of the salt, the blood level of penicillin after administration of intramuscular potassium penicillin V is high initially, as expected; however, the values soon decrease to below the therapeutic limit because of rapid excretion. It is remarkable that calcium penicillin V, of which only 1 per cent is soluble, does not remain longer in the blood; however, the degree of solubility is obviously not low enough so that this preparation may remain in desired proportions. As shown in these investigations, calcium penicillin V may not be included with the depot preparations.

The presumed complete transformation of the acid penicillin V into a soluble alkali penicillin at the point of injection could not be ascertained by these experiments. On the contrary, it was surprising to find a positive depot effect much resembling the depot effect of procaine penicillin G. However, obviously a certain part of acid penicillin V forms a salt with the alkalis present in the tissues and is very quickly absorbed, since the high water-solubility gives a high initial curve, which is typical for the potassium salt. Nevertheless, a sufficient part of phenoxymethyl penicillin remains in the tissue in an insoluble form, securing a sufficient depot effect.

It is seen from these experiments that the absorption of the different salts of penicillin or of the acid depends on the solubility of the substance. This is clear in every case, from the almost completely soluble potassium penicillin V, to low-soluble calcium penicillin V, to acid penicillin V, of which only 0.05 per cent is soluble. Therefore, the curve of the blood level shows definite parallels to those in our urinary excretion experiments with different soluble penicillin salts by intramuscular administration.<sup>16</sup>

The excretion values of procaine penicillin G and of acid penicillin V demonstrated in the present report suggest a discussion of the distribution of both substances in the tissue. Although by planimetric evaluation the blood levels are equal during the whole test time (quotient acid penicillin V: procaine penicillin G = 1.12), urine excretion values show significant differences. The total excretion of procaine penicillin G is 81 per cent, but of acid penicillin V it is only 54 per cent, giving a 0.66 proportion between acid penicillin V and procaine penicillin G. Since during passage from the blood to excretion through the kidney, the intervening tissue may destroy the penicillin molecule, it must be indirectly concluded that phenoxymethyl penicillin diffuses more into the tissues than benzylpenicillin. Since in almost all cases the level in the tissue—and not the blood level—is important, new possibilities are seen for the practical application of the acid penicillin V. These experiments also suggest that the findings of Dost<sup>18</sup> with regard to amount of distribution after intravenous application may also be true for intramuscular administration, as has been discussed also by Hitzenberger and Spitzky.<sup>19, 20</sup> With regard to the local compatibility of phenoxymethyl penicillin, we may confirm that objective or subjective reactions have never occurred.

#### CONCLUSION

Proceeding from allergic manifestations that may be caused by cation constituent of depot penicillins, acid penicillin V, as a molecule without an allergenic cation constituent, was investigated with respect to its efficacy in the case of parenteral administration. Highly soluble penicillin V salts and/or calcium penicillin V produce

no depot effect in rabbits with 10,000 units/Kg., but the basic substance of this class of compounds, i.e., phenoxymethyl penicillin (acid penicillin V), must be classified among the depot penicillins according to our results obtained with rabbits and human beings. Unlike what occurs with procaine penicillin G, a high initial peak is attained by this substance, which is explained by the partial transformation of acid penicillin V into a highly soluble salt at the site of injection. The amount of absorption depends on the solubility in water, and the same conditions were found to prevail as were observed at study of urinary excretion carried out in the course of earlier experiments. The differences found between acid penicillin V and the procaine penicillin G with respect to percentage of the urinary excretion, planimetric values of blood level being equal, are explained by the difference in distribution of the two substances in the tissues.

#### BIBLIOGRAPHY

1. HERRELL, W. E., AND NICHOLS, D. R.: Procaine penicillin G (Duracillin): a new salt of penicillin which prolongs the action of penicillin, *Proc. Staff. Meet., Mayo Clin.* 22:567, 1947.
2. ROBINSON, J. A.; HIRSCH, H. L.; MILLOFF, B., AND DOWLING, H. F.: Procaine penicillin: therapeutic efficiency and a comparative study of the absorption of suspensions in oil plus aluminum monostearate and of an aqueous suspension containing sodium carboxymethyl-cellulose, *J. Lab. & Clin. Med.* 33:1232, 1948.
3. LEPPER, M. H.; DOWLING, H. F.; ROBINSON, J. A.; STONE, T. E.; BRICKHOUSE, R. L.; CALDWELL, E. R., AND WHELTON, R. L.: Studies on hypersensitivity to penicillin. I. Incidence of reactions in 1303 patients, *J. Clin. Invest.* 28:826, 1949.
4. SCHEIFFART, F., AND FENGER, W.: Wesen und klinische Bedeutung der Arzneiallergie, *Arztl. Wchnschr.* 11:73, 1956.
5. LONG, P. H.: Fatal anaphylactic reactions to penicillin. *In: Antibiotics Annual 1953-1954*, New York, Medical Encyclopedia, Inc., 1954, p. 35.
6. BELL, R. C.: Sudden death following injection of procaine penicillin, *Lancet* 266:13, 1954.
7. KÄRCHER, K. H.: Die Penicillinallergie als ernstzunehmender Therapieschaden, *Medizinische* 33/34:1089.
8. MÜCKTER, H.; JANSEN, E.; SOUS, H.; KRÜPE, W.; QUAST, A., AND KELLER, H.: Darstellung und Eigenschaften eines neuen Depotpenicillins, *Arzneimittelforschung* 4:487, 1954.
9. AHNEFELD, F. W.: Neopenyl—ein procainfreies Depotpenicillin, *Med. Klin.* 49:1480, 1954.
10. GRANATEK, A. P.; MINOR, W. F., AND GOTTSTEIN, W. J.: Antihistamine salts of penicillin. II. Preparation and properties of the penicillin G salt of N,N-dimethyl-N'-benzyl-N'-( $\alpha$ -pyridyl) ethylenediamine, *Antib. & Chemo.* 4:1159, 1954.
11. WILLIAMS, J. A.; MEYNELL, M. J., AND WATSON, A. B.: Benethamine penicillin. A study of its use in a clinic for septic hands, *Brit. M. J.* 1:716, 1956.
12. ELIAS, W. F.: Parenteral penicillin depot preparations, *Pharmacy Int.* 10:12, 1956.
13. PETERSEN, H., AND ALLIES, F.: Zur Antihistamin-Penicillintherapie im Kindesalter, *Deutsche med. Wchnschr.* 81:1961, 1956.
14. FENNER, O.: Ein Antihistamin Penicillin mit protrahierter Depotwirkung, *Arzneimittelforschung* 6:719, 1956.
15. MACCORQUODALE, D. W.; DEROSE, A. F.; MICHAELS, R. J.; WESTON, A. W., AND RICHARDS, R. K.: Hydrabamine penicillin G, a new penicillin salt of low solubility. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 133.
16. GIOVANNINI, M., AND KRAUSHAAR, A.: Die Ausscheidung verschiedener Penicilline mit dem Harn, *Arzneimittelforschung* 8:86, 1958.
17. BAUER, K.; HILTMANN, R.; SCHMID, J., AND AUHAGEN, E.: Neue, kristallisierte Penicillinsalze und ihre Prüfung auf Depotwirkung am Kaninchen, *Med. u. Chemie* 5:25, 1956.
18. DOST, F. H.: *Der Blutspiegel*, Leipzig, Verlag Thieme, 1953.
19. HITZENBERGER, G., AND SPITZY, K. H.: Verteilung und Elimination von Phenoxymethyl-Penicillin (Penicillin V) als Maß für seine therapeutische Wirksamkeit, *Arzneimittelforschung* 7:1, 1957.
20. SPITZY, K. H.: Über die Bestimmung des Verteilungsvolumens der Antibiotika im Körper am Beispiel von Penicillin und Novobiocin, *Gior. di microbiol.* 2:379, 1956.
21. ELIAS, W. F., AND MERRION, H. J.: Some observations on penicillin V. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, p. 510.
22. BRUNNER, R., AND GIOVANNINI, M.: Das Dibenzyllaethylendiamin-di-Penicillin V (DBED-Penicillin V). Seine Anwendung in der oralen Therapie, *Wien. med. Wchnschr.* 106:243, 1956.
23. GIOVANNINI, M., AND BODSCH, H.: Das Dibenzyllaethylendiamin-di-Penicillin V (DBED-Penicillin V). Seine Anwendung in der intramuskulären Therapie, *Wien. med. Wchnschr.* 106:600, 1956.
24. DOUJAK, P. H., AND SPITZY, K. H.: 3Tage Depot mit DBED-Penicillin V als Basis für die perorale Therapie mit Penicillin V, *Wien. med. Wchnschr.* 107:470, 1956.

# Control of the Common Cold by Autogenous Vaccine or by Antibiotics

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Work with autogenous vaccines for the common cold led the way to the work done with antibiotics. In the early 1930's, requests for vaccine treatment were made by clerks of the Birkenhead health department who had been chronic sufferers from the common cold. These were refused on the ground that, since colds were due to a virus, the treatment would necessarily be useless.

One woman, however, who had had one continual cold for several winters, was very persistent in her appeals for a vaccine, although she was told repeatedly that no benefit could follow. Ultimately a vaccine was made from her saliva, and she was plainly told that it was being given to her only to prove its uselessness. She had weekly injections throughout the next winter and had no cold. Although told that was a pure coincidence, she presented herself, with several others, for further vaccine at the end of the next summer. Autogenous vaccines were not refused, but it was strongly explained to all that no possible effect could be expected. All experienced a winter free from colds, although some had the prodromal stages.

It was felt that this continued result was a strange coincidence but nothing more. For some years a small but steady stream of clerks and policemen were treated, more or less unwillingly, by the laboratory. Although no special investigations were possible, it gradually became apparent that during a period of some 20 years, in which 60 or 70 persons were vaccinated, almost the only failures were some cases of long-standing, where it was practically certain that degenerative changes had taken place in the mucous membrane. In a few cases the improvement might be explained on psychological grounds, but the series was becoming too large for this explanation to be sufficient.

As a result of these and other findings, the opinion was formed that the common cold might be due to two factors—the virus, whose function is essentially depressive, and an invasion by the patient's own normal but heterogeneous secondary organisms, which are usually present in a state of equilibrium, becoming active only as a result of the patient's depressed resistance caused by the virus. If this explanation was correct, these volunteers, experiencing the prodromal stage for a day or two, still kept on taking their colds as far as the virus was concerned, but were immunized against the effects of their own organisms. If patients could be protected against these, the virus could be at its worst, yet cause only slight nasal inconvenience. In support of this explanation was the fact that penicillin lozenges were by then known to clear up colds in certain persons only, being useless in others—depending presumably on the sensitivity of the patient's own flora. Similar support was given by the unreliable results of stock vaccines, which were possibly of value only when the stock strains were the same as the patient's own.

In the spring of 1955 it was decided to carry out an investigation in a large factory. It was agreed that 200 volunteers should be asked for, of whom 80 would be controls. This entailed giving a series of injections to all 200, of whom 120 received an autogenous vaccine and 80 carbol saline only. One hundred and nine of the vaccinated and 75 of the controls completed the investigation.

No attempt was made to identify individual organisms. This had been tried years before, with very diverse results. In this series *Streptococcus viridans* appeared to predominate in about four fifths of the volunteers. Even these, however, were heterogeneous.

#### ADMINISTRATION OF VACCINE

The first dose was invariably 0.1 ml. Controls were dealt with in exactly the same way as those receiving the vaccine itself. A sheet of instructions was given to each volunteer, asking him to report to the health center on the firm's premises if any reaction, however trivial, was experienced. Doses were repeated at intervals of a week. The second dose was 0.3 ml., and the third and subsequent doses, 0.5 ml., but when there was a reaction to the initial dose, the next was reduced to 0.2 or 0.1 ml. There was local redness at the site of inoculation in 71 of the vaccinated volunteers (65 per cent) and in 9 (12 per cent) of the controls. This difference between vaccinated persons and controls was kept secret and, in fact, denied when any hints or surmises were made. The area of redness was usually  $\frac{1}{2}$  to 1 inch in diameter, was mildly tender for a day or two, and then subsided. In a few of the vaccinated persons an area of redness developed several inches across. The larger reactions were usually in volunteers who were definitely subject to colds.

Throughout this and the antibiotic investigations, which were carried out on exactly the same lines and will be described later, patients were asked to report at the medical centers of the works concerned when they felt the prodromal stages of a cold. These were noted as "incidents," and records were kept at the factory of the numbers of incidents in vaccinated and control persons that went on to become full colds. An incident was regarded as having aborted if symptoms were strictly confined to the initial stages, i.e., up to a nonpurulent running nose for two days (rarely three) with no further symptoms and no apparent toxicity. This stage corresponds to what, it is suggested, may be due to the virus itself, and therefore unaffected by either vaccine or antibiotics. Assessment of results was made by the staff of the factory health centers, who did not know which volunteers were controls in either the vaccine or the antibiotic tests.

To sum up the results of the vaccine experiment, the five months of the trial were considered separately and the number of volunteers under trial for each was totaled. This gave a total of 371 volunteer months for vaccinated persons as against 375 volunteer months for controls. In the vaccinated there were 13 colds (4 per cent); in the controls, 77 colds (21 per cent).

Of 25 cold-prone volunteers (known by their factory medical history), 48 volunteer months in vaccinated persons produced only one cold (2 per cent) as compared to nine colds out of 36 volunteer months in the controls (25 per cent). Time lost through absence showed a similar trend.

These results were regarded as highly encouraging and suggest that, in fact, the essential function of the virus (or viruses) might well be that of an activator, which, by depressing resistance, relatively enhances the patient's own nasopharyngeal organisms.

However, autogenous vaccines are impracticable except for individual cases or on a small research scale. It was decided therefore to test the effect of short-term controlled antibiotic therapy, after ascertaining the sensitivity of the patient's

pharyngeal flora before the winter began. If vaccines had given a degree of active immunity, then suitable antibiotic tablets given to suck at the beginning of the prodromal stage might well give sufficient passive protection by depressing the pharyngeal flora temporarily to correspond to the depression of the patient's resistance. To avoid risk of producing insensitive strains of bacteria or of disturbing permanently the balance normally maintained between patient and pharyngeal flora, it was decided to study the effect of brief local application only. After preliminary trials had shown that the sucking of lozenges did, in fact, reduce the numbers of pharyngeal flora and that this effect was temporary, invitations to cooperate were sent out through the medical officers concerned to some of the larger factories in the neighborhood. Altogether 1043 volunteers took part, of whom 919 completed the investigation.

The test was carried out on exactly the same lines as the previous (vaccine) investigation. Although sensitivity tests were done on all, every fourth volunteer was designated a control and received an inert tablet. All tablets were issued from the laboratory, labeled by number only. As previously, assessment was made by the factory health staff, who did not know which tablet was the inert control.

#### THE TABLETS

In so few cases did penicillin and erythromycin give the best inhibition rings that it was not worth while considering them for the test itself. Streptomycin was little better. Chloramphenicol gave the best ring in many cases, but its bitterness ruled it out entirely. Those left were all preparations of tetracycline in some form. The sensitivity rings tended to differ, and it was deemed advisable to treat each as a separate entity. Our experience was largely with oxytetracycline.\*

#### DOSAGE

Dosage was purely empirical. To avoid the chance of ill effects, the very small dosage of 2 tablets a day for two days was tried. It became clear that the original cautious dosage was in some cases too small. In the later stages of the trial, a dosage of 2 tablets a day for not more than three days was allowed when required. There were no reports of untoward results of the increased dosage. Another factor that became obvious was that, to have maximal effect, treatment should be begun on the day that the earliest symptoms are noticed.

#### RESULTS

The proportion of volunteers with prodromal symptoms who went on to a full cold was consistently much lower in the treated than in the control groups. Including all who were seen, i.e., those who reported an incident a day or two late, there were 43 colds among 432 volunteers who received the antibiotic tablets (i.e., 10 per cent) as compared to 87 in 338 controls who received an inert tablet (26 per cent). Of those who reported an incident on the first day, the proportion was 4 to 26, i.e.,  $6\frac{1}{2}$  times as many among controls as among those who received antibiotics. In two places—one a metal factory and one a large office—there were actually no full colds among 164 volunteers who received antibiotic tablets as compared to 47 colds in 86 controls. Included in the total was a boys' boarding school where, as one

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\* The trade name of Chas. Pfizer & Co. for oxytetracycline is Terramycin.

would expect, the different age group gave a different series of percentage ratios. Even here, however, there was a drop of 4 to 1 between the control and treated groups—from 64 to 17 per cent—which it is felt is definitely encouraging for young boys. Among the treated adult volunteers (excluding latecomers), 14 colds developed in 533 volunteers (2.6 per cent) compared with 73 colds in 316 volunteers in the controls (23.1 per cent), a ratio of 1 to 9.

The small dosage of antibiotics used had no permanent effect on the sensitivity of the pharyngeal bacteria or on the proportions of the various species, although some volunteers had irritation of the throat or tongue.

During the past winter, a further series of 1120 volunteers were investigated on exactly parallel lines. The results were similar, i.e., including all who were seen, 6.2 colds/100 volunteers in the treated and 22.7/100 in the control group. For 304 volunteers, latecomers being separately assessed, the figure was 4.8 per cent.

#### PRACTICAL APPLICATION OF THE FINDINGS

In industry, when a factory has a medical center, the antibiotic method is extremely simple and consists of the following: (1) For each worker, one sensitivity test is done by a laboratory at the beginning of the common cold season. (2) The appropriate antibiotic tablets are substituted for whatever palliative has been used in the individual factory concerned. (3) Care is taken not to overdo the antibiotic and that no objection is raised by the patient's own physician.

Apart from industry: (1) Chronic cold sufferers: The method of choice here is the autogenous vaccine as giving active immunity. A short course of minimal oral antibiotic may, if necessary, be given to prevent development beyond the prodromal (virus) stage, but this may not be necessary once immunity has developed. The sensitivity test can be recorded when the vaccine is made in case it is required. (2) The "casual" cold: This does not warrant a vaccine and should respond to oral antibiotics on the lines indicated. In industry, when a medical center is available, sensitivities can easily be recorded in the early autumn for use throughout the ensuing winter. There is, of course, nothing to prevent practitioners doing the same with patients, but as these do not usually report at the beginning of a cold, the early opportunity is likely to be missed. It has been found that although there is a considerable degree of constancy in the mouth flora, it is better to repeat the sensitivity test each autumn. (3) Influenza: Many practitioners have noticed that after treating some early cases of influenza with systemic antibiotics on purely empirical grounds, the incidence and severity of complications have been reduced. It is perfectly logical to assume that if the cold virus is essentially an activator, the influenza virus may be the same, albeit on a different scale. The continued use of systemic antibiotics as a prophylactic is, of course, to be strongly condemned as defeating its own purpose, through the production of resistant bacteria, but their administration for a few days in an emergency, like an imminent attack of influenza, is open to no such objection. As a sensitivity test can readily be ascertained overnight, attacking the nasopharyngeal flora in advance by the appropriate antibiotic may well prove to be a valuable method of reducing the effects, even in cases of influenza.

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# Chemoprophylaxis in Asian Influenza for Patients with Chronic Pulmonary Disease

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The pandemic of Asian influenza, although considered generally mild, brought severe sickness, or even death, to many thousands. A high percentage of these unfavorable cases occurred in persons with chronic pulmonary diseases or other pre-existing ailments.<sup>1-7</sup> This increased risk of serious illness in certain special groups became evident from the very beginning of the epidemic. In July, 1957, at the Fourth National Boy Scout Jamboree, the incidence of influenzal pneumonia among the boys and adults with bronchial asthma was more than three times that in others.<sup>8</sup>

The favorable results from chemoprophylaxis in Asian influenza for my patients with chronic pulmonary disease, as well as the lack of other reports on preventive chemotherapy during the epidemic, gave rise to this communication.

## MATERIAL AND PURPOSE OF THE STUDY

Between September, 1957, and March, 1958, 96 of some 200 patients with recurrent or chronic respiratory infections had an acute febrile illness with the clinical picture of influenza. Most of these episodes occurred in October or November, 1957, some of them in January or February, 1958, and a few at some other time.

Seventy-five of the 96 patients were children and 21 were adults. Forty-four had chronic bronchitis; 48, infectious asthma; and 4, moderately advanced, inactive tuberculosis. Two thirds of the group had an associated chronic upper respiratory infection. About 30 per cent of the children and 40 per cent of the adults had a history of one or more attacks of pneumonia; nearly 50 per cent of those older than 40 had had epidemic influenza in 1918. The type of the underlying chest disorder ranged from recurrent bronchiolitis in infants to extensive bronchiectasis or advanced emphysema in middle-aged or elderly patients.

All of this group had been under continuous or intermittent antibiotic treatment to prevent or control chronic bronchopulmonary disease, some for as long as 10 years.<sup>9-15</sup> This ailment usually originates from lung-damaging respiratory episodes, and progresses further when subsequent bacterial exacerbations add new insults. Therefore, in these patients, immediate and adequate antibiotic treatment of Asian influenza was held important, not only because of the imminent danger, but also as an essential part of the program of rehabilitation.

Although I did not expect that antibacterial agents would affect the viral phase of influenza, from my previous experience I anticipated that they would suppress the bacterial component of the illness, thus mitigating its course.<sup>9,11,12,14</sup>

## TREATMENT

Eighty-three of the 96 patients were started on chemoprophylaxis on the first day of influenza, 11 on the second day, and 2 on the third.

The antibiotics most frequently used were penicillin and tetracycline. To reduce the risk of serious complications from treatment, injections of penicillin were avoided whenever possible. During the seven months of this project, only 11 of the 96

patients received penicillin injections at some time, always together with streptomycin. In my previous investigations, this combination had proved particularly effective in acute respiratory infections, and it has been recommended for severe cases of Asian influenza in which gram-positive and/or gram-negative bacteria may be secondary invaders.<sup>4, 16</sup>

Therefore, it seemed reasonable to assume that the oral administration of penicillin in combination with tetracycline also would suppress most completely a variety of pathogens and potential pathogens. Both influenza and chronic bronchitis are associated with a mixed bacterial flora in which pneumococci, *Hemophilus influenzae*, staphylococci, and streptococci play varying and unpredictable parts. Moreover, such multiple therapy made it possible to give therapeutic but moderate dosages of penicillin and tetracycline and thus to avoid possible hazards from excessive dosages of either.

Laboratory tests thus far have not proved a reliable guide for antimicrobial treatment of respiratory infections, nor do they necessarily reflect the clinical therapeutic response.<sup>17-23</sup> Therefore, and also because they would have been unfeasible in ambulatory patients during an epidemic, the selection of antibiotics was made empirically.

The character and extent of the underlying chronic disease, the severity of the acute illness, the patient's response to treatment, but also his tolerance of the antibiotic—all these factors required individualized consideration. Another problem in antibiotic treatment in general, the expense involved, was of minor importance in the present study because of the generous supplies of free antibiotics provided.

In almost all of the children, penicillin V, particularly its potassium salt,\* was used because its higher acid stability permits regular treatment without regard to food intake.<sup>14, 15, 20</sup> The total daily dosage varied between 750 mg. (1,200,000 units) and 2 Gm. if this antibiotic was given alone. Penicillin G taken on an empty stomach was given in about the same amounts. The dosage was reduced at most by one half for multiple antibiotic treatment. Tetracycline† was given alone or with penicillin in dosages of not less than 300 mg./day in young children and not more than 1.5 Gm. in adults. Patients who had been using penicillin aerosol as maintenance therapy at home<sup>13, 24</sup> continued this type of topical administration. Eight patients received chloramphenicol at some time for brief periods, but none of the newer antibacterial drugs was used.

As a rule, multiple antibiotic therapy was given to patients whose chest ailment was incompletely controlled before the influenza attack and to those whose acute illness appeared severe. Patients who in the beginning took a single antibiotic continued it if their temperature became normal within 48 hours and respiratory symptoms subsided. Otherwise, the second antibiotic usually was added to, but in some cases given instead of, the first one. Of course, patients who had experienced pronounced ill effects from penicillin received tetracycline, and vice versa. In these and other instances in which a single antibiotic was used, penicillin usually was combined with triple sulfonamides, and tetracycline with sulfamethoxypyridazine.‡

It is obvious that the changes made in antimicrobial agents during the emergency

\* The trade name of Eli Lilly & Co. for penicillin V is V-Cillin; and for penicillin V potassium, V-Cillin K.

† The trade name of Lederle Laboratories Division, American Cyanamid Co., for tetracycline is Achromycin; and for sulfamethoxypyridazine, Kynex.

of an epidemic do not necessarily reflect the comparative value of penicillin, tetracycline, and the combination of both. At the beginning of the illness, oral penicillin was given to 47 patients, tetracycline to 23, and both antibiotics to 26. In the course of the illness, about 70 per cent of the penicillin group received tetracycline, mostly in addition to penicillin. In 50 per cent of the tetracycline group, that antibiotic was supplemented with or replaced by penicillin. In 60 per cent of the patients treated with penicillin and tetracycline, a change from tetracycline to chloramphenicol was made or parenteral penicillin with streptomycin was given in addition to the previous medication.

Generally speaking, large dosages of penicillin, especially potassium penicillin V, achieved subsidence of fever, but a persistent bronchitis cleared up more quickly and more completely with tetracycline. On the other hand, the use of tetracycline as the sole antibiotic, even if it was effective, was limited because of more or less pronounced side reactions from large dosages.

Patients who had been taking antibiotics continuously before contracting influenza continued this treatment after their recovery. Those who received chemoprophylaxis only for the acute illness continued it for at least two weeks after their complete recovery. Any further important respiratory episode was again thoroughly treated with antibiotics.

As the epidemic abated and serious illnesses declined, the relative value of penicillin and of tetracycline in long-term antibiotic treatment of chronic respiratory infections became more distinct. As before, penicillin proved an excellent maintenance therapy, and in many instances it suppressed febrile episodes.<sup>11-15, 24</sup> The major advantage of tetracycline remained its effectiveness in exacerbations, especially when penicillin failed. These observations confirm those of others.<sup>17-23, 25</sup>

In influenza, as well as in chronic pulmonary diseases, factors other than infection may predominate and account for "antibiotic failures." Therefore, adjunctive medication played an important role in this study. Thirty-one of the patients with infectious asthma had been taking anti-inflammatory steroids, mostly prednisone,\* continuously or occasionally for relief of bronchial obstruction.<sup>15</sup> When taken ill with influenza, all of them continued combined antibiotic-steroid treatment. Five additional patients were given hormonal treatment for brief periods to improve bronchial drainage. For the same reason, expectorants and bronchodilators were given liberally, but cough-suppressing and drying medicaments, such as codeine and antihistamines, were not used in any case.

Under the type of treatment just outlined, emergency care during and after the epidemic was not often needed. Between September, 1957, and March, 1958, only 1 of the 96 patients had to be hospitalized and only 9 required a house call. On the other hand, most patients were seen in the office within a week after the onset of influenza, and the remaining within two weeks, chiefly to rule out any sequelae to their illness.

## RESULTS

Eighty-six of the 96 patients made a prompt and uneventful recovery from the influenza. The temperature fell to normal by the third day, often within 24 hours, and did not rise again; the general fatigue ceased before the end of the second week, and usually within one week. Pronounced cough was present in most of these patients with chronic respiratory ailments, but this symptom subsided after one or at most

\* The trade name of the Schering Corp. for prednisone is Meticorten.

two weeks. Asthmatic symptoms rarely flared up, and none of the patients with chronic asthma went into status asthmaticus.

In 6 patients, Asian influenza caused a more protracted illness. In 4 others, some complication developed, but it was severe in only 1. This was a case of pneumonia in an adult, the only patient of the 96 who was hospitalized. In this patient and 3 other adults, all with long-standing chest ailments, the influenza caused a prolonged exacerbation, in spite of intensive chemotherapy. However, no serious, life-threatening illnesses occurred, and all patients regained their previous health status after two or three months.

Among the children who received chemoprophylaxis, 3 had a mild and transient otitis media catarrhalis. In 2 children, both with a previous history of multiple attacks of pneumonia, the influenza was followed by stubborn bronchitis, which cleared up completely within three weeks. None of the 75 children, of whom 9 were less than 5 years old and 39 had a history of infectious asthma, developed a clinically or roentgenologically detectable pneumonia.

The relatively mild effect of the influenza on this group as a whole is also apparent from the short average duration of disabling sickness. The 12 employed persons were absent from work an average of six days, and the 66 children more than 5 years old lost an average of four days from school.

Apart from the immediate favorable results of early chemotherapy, this treatment proved beneficial also during the critical postepidemic months. Some patients with advanced pulmonary disease had a "bad winter," as they had had before. None of them, however, experienced a serious illness, even when, early in 1958, mortality from influenza and pneumonia among the general population reached a new high.

Absence from school reflects the prevalence of illness in the community.<sup>26</sup> Respiratory sickness is the major cause of lost school days, even in normal times, and accounts for the bulk of absenteeism during epidemic-like illnesses. Therefore, the comparison of absenteeism in susceptible children receiving chemoprophylaxis for respiratory infections with that of untreated, susceptible children has in the past provided a fair estimate of the value of such protective treatment.<sup>11, 12, 14, 15</sup>

With the cooperation of health and school authorities, figures were obtained on absences during and after the epidemic of Asian influenza in the general school population, in 84 "treated" children, and in 70 untreated "controls." Both the treated and the control groups consisted of children with recurrent or chronic respiratory ailments who attended various schools in the community.

The treated group included all school children of the present study who had an attack of influenza and received chemoprophylaxis for any respiratory infection. The 70 controls were treated by other physicians, if at all, with generally accepted methods.

Details of the analysis will be published in a forthcoming paper. For the purpose of the present study, the following figures seem most pertinent.

At the height of the epidemic, during the month ending Oct. 21, 1957, total absenteeism among the general school population approximated 20 per cent of possible school days. The corresponding figures were 17 per cent in the treated group and 28 per cent in the controls. During the period Sept. 4, 1957, through March 21, 1958, absenteeism was 10 per cent in the general school population, 8 per cent in the treated group, and 13 per cent in the controls. During the epidemic, as well as in its wake, prolonged disabling sickness was far more prevalent in susceptible children treated conventionally than in those receiving antibiotic prophylaxis consistently. By March, 1958, absenteeism had exceeded the general average in two

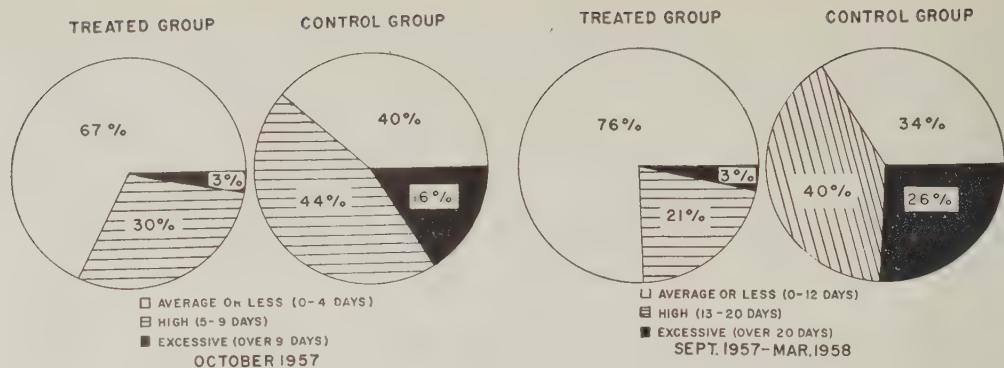


FIG. 1. Percentage distribution of average, high, and excessive school absenteeism in 84 children with recurrent or chronic bronchitis or infectious asthma, receiving chemoprophylaxis, and in 70 children with a similar history, treated conventionally, is shown. On the left, data at the height of the Asian influenza epidemic in October, 1957, are given for both the treated and control groups; on the right, data for both the treated and control groups are shown for September, 1957, to March, 1958, during and following the epidemic.

thirds of the controls, but in only one fourth of the treated group. Absences totaling more than four school weeks had occurred nine times as often in the controls as in the treated group (fig. 1).

Although no specific information on illnesses in the control group was usually available, several cases of severe sickness, including pneumonia, suppurative otitis media, and nephritis, were reported.

#### SIDE EFFECTS FROM TREATMENT

Five patients experienced mild or moderately severe urticaria, 4 of them while taking large dosages of potassium penicillin V and 1 after an injection of penicillin. In all 5, this untoward reaction subsided within two days after discontinuing penicillin, replacing potassium penicillin V with regular penicillin V, or administering antihistamines and prednisone. Four of the 5 patients were able to take smaller dosages of oral penicillin again at a later date without any side effect.

Eleven patients taking tetracycline had pronounced diarrhea, with or without rectal and vaginal inflammation. In none of them did this side reaction result in serious or disabling sickness. Discontinuance of tetracycline or reduction of the dosages overcame these effects in some cases. In others, oral nystatin or topical administration of nystatin with hydrocortisone usually achieved prompt relief, but in 2 the untoward symptoms persisted for two weeks.

Prednisone caused overstimulation, polyuria, and rapid gain in weight in 4 of the 36 patients who received hormonal treatment, but in all instances these side effects could be controlled easily. As in previous experience,<sup>15</sup> spread of infection by steroids did not become evident. This potential risk probably was avoided because prednisone was always given under antibiotic cover.

#### BACTERIOLOGICAL STUDIES ON SUPERINFECTIONS AND *Staphylococcus* CARRIERS

As mentioned before, in none of the 96 patients receiving chemotherapy for influenza did a clinical infection of overwhelming treatment-resistant character occur.

The 1 patient who developed pneumonia while taking penicillin and tetracycline was a sickly, elderly woman with a history of multiple attacks of pneumonia and chronic bronchiectasis. Although the fever subsided in less than one day, she was

hospitalized because of pronounced respiratory distress, atelectasis being suspected. Sputum cultures disclosed *Diplococcus pneumoniae* and *Klebsiella pneumoniae*, and treatment was changed to parenteral penicillin with streptomycin, and oral chloramphenicol. For improving bronchial drainage, prednisone and aerosol therapy with bronchodilators and mucolytic agents were given. The patient recovered clinically from the acute illness after 10 days' hospitalization, although bacteriologically *K. pneumoniae* persisted for several weeks in the sputum. Since this organism was a rather frequent secondary invader in pulmonary infections even before the antibiotic era, its presence in this particular case may or may not be attributable to antibiotic prophylaxis.

Nose, throat, and sputum cultures also were taken from 18 patients whose respiratory infection was clinically controlled only incompletely in spite of chemotherapy. Regardless of which antibiotic or antibiotics were used, in none of these cases were hemolytic streptococci, staphylococci, *H. influenza*, or *K. pneumoniae* found. In 1 case *D. pneumoniae* was present but not predominant. The bacterial flora usually consisted of such organisms as alpha streptococci and *Neisseria catarrhalis*, which are of questionable clinical significance. In a few instances, coliform bacteria were present, as they often are in patients receiving chemotherapy, whether or not the infection is clinically controlled. At any rate, with the one possible exception in the case of pneumonia, no bacteriological "breakthrough" paralleled by severe illness was observed. A lack of correlation between the bacteriological and clinical picture, especially in antibiotically treated patients, has also been reported in recent publications.<sup>18, 21-23, 27</sup>

In the spring of 1958, saliva samples from 32 children, including those who had been given chemoprophylaxis for Asian influenza, were examined. These bacteriological studies were done by the Eastman Dental Dispensary in connection with controlled investigations on the reduction of tooth decay in children receiving long-term antibiotic therapy. The bacterial count indicated a remarkable reduction in the aerobic flora. Sensitivity tests did not demonstrate an overgrowth of penicillin- or tetracycline-resistant *Micrococcus pyogenes* variants.

#### COMMENTS

Viruses, particularly those of influenza, often cause profound damage to the respiratory system and render it susceptible to secondary bacterial invasion.<sup>28</sup> In the majority of cases, bacterial pneumonias are preceded by viral respiratory infections.<sup>28, 29</sup> Laboratory tests are not reliable for differentiating between a self-limited viral illness and a potentially serious bacterial illness.<sup>21, 30</sup> Therefore, the relative importance of viruses and bacteria cannot be determined in the early stage of influenza. However, bacterial infection should be treated antibiotically in its beginning, because delay of therapy reduces its effectiveness and may result in an overwhelming infection.

During the epidemic of Asian influenza, failure to give antibiotics in time apparently proved fatal to many,<sup>7</sup> and available data suggest that very few among those who died had received these drugs at the beginning of their illness.<sup>5, 31</sup>

For these reasons, immediate chemotherapy in influenza is definitely indicated for persons with pulmonary diseases or other ailments. Such treatment also seems advisable for others if, by their history, severe sickness tends to follow even mild respiratory illnesses.

The responsible authorities were aware of the increased risk of serious sickness

in susceptible persons and of the benefit such persons might obtain from early antibacterial management. While strongly advising against this treatment as primary therapy for influenza, they made possible exceptions to this rule for persons with chronic respiratory disease and other pre-existing ailments.<sup>7,16</sup>

In spite of the general view that antibiotics are without any value as primary treatment of influenza and other viral respiratory infections, certain observations favor such prophylactic therapy for possible prevention of secondary bacterial complications.<sup>21, 29, 32-34</sup> In fact, during the Asian influenza epidemic in the Philippines, early treatment with tetracyclines apparently did shorten the febrile phase and reduce fatalities.<sup>35</sup>

An increasing number of observations supports the view that antibacterial therapy can be effective in acute and chronic respiratory infections, regardless of their bacteriological aspects.<sup>18-23, 36</sup> Ritchie's<sup>37</sup> recent studies indicate that even in the "common cold," disabling sickness can be shortened by suppressing the bacterial component.

The results of the present investigation strongly suggest that, at least in the vulnerable, early antimicrobial therapy is valuable in reducing the risk of serious and protracted sickness in influenza. Naturally, in ambulatory patients with an illness that killed some of its victims within one day, a double-blind test could not be used. However, some evidence has been submitted here that indicates that the influenza inflicted less harm on the antibiotically treated patients than was to be expected.

Disabling sickness in the children was shorter than in a comparable untreated group. The adults lost an average of six days from work, against the 10 to 14 days observed, for example, in Fry's<sup>38</sup> group of the general population. Only 1 case of pneumonia occurred among the 96 patients receiving chemoprophylaxis during the entire observation period, which included the "second wave" of Asian influenza. Even in previously healthy military personnel, the incidence of pneumonia immediately after influenza approximated 3 per cent.<sup>39</sup> This figure went up to 15 per cent in adults and children with bronchial asthma.<sup>8</sup>

The prime reason for the official recommendation not to use antibiotics prophylactically in Asian influenza was the possible risk involved in such therapy.<sup>16</sup> Evidently, in susceptible persons to whom this rule did not apply, the dangers from complications of influenza were held more imminent than those from antibacterial treatment.

Among the 96 patients of this study who belonged to that susceptible category, chemoprophylaxis, even with additional steroids, caused few complications, none of them serious. There was no perceptible increase of drug-resistant strains of microorganisms or of *Staphylococcus* carriers. This observation confirms the results of similar investigations, including those on prolonged antibiotic prophylaxis.<sup>12, 15, 17, 18, 22, 23, 25, 33, 34, 36, 37, 40, 41</sup>

The magnitude of the actual danger of increasing *Staphylococcus* infections and the assumption that this rise is caused by the increasing use of antibiotics have been questioned by several authors.<sup>42-44</sup> At any rate, the phenomenon of treatment-resistant staphylococci is chiefly a hospital problem;<sup>45</sup> it cannot reasonably be used as an argument against a more liberal use of antibiotics in ambulatory patients when such treatment can ease human suffering.

The prevalent tendency to minimize the potential benefits and magnify the possible dangers from chemoprophylaxis has been a hindrance to large-scale investigations. Controlled studies during the Asian influenza epidemic might have demonstrated conclusively whether or not early and adequate antibacterial treatment is

valuable and how great the actual risks from such therapy are. Greene's and Hair's<sup>39</sup> report, for example, shows that an evaluation of early antibiotic treatment was not even considered, because of fear of complications.

The wisdom of using several antibiotics alone or in combination, by trial and error, may be questioned. However, as long as there is no reliable or feasible method for determining the pathogens actually responsible and for predicting the most effective and least harmful treatment, selection of antibiotics will have to be made chiefly on empirical grounds.

It required many years and much research to gather even limited knowledge on optimal chemotherapy in tuberculosis. Obviously, the "ideal" antibiotic for any infection—that is, the most effective, the best tolerated, and the least expensive—still has to be discovered. Of prime importance in nonspecific respiratory infections, as in tuberculosis, is the earliest possible suppression of the infectious process by all available means.

Even if the results of this study may not be conclusive, they seem convincing enough to warrant further investigations on the pros and cons of chemoprophylaxis in viral infections, particularly in influenza. Sooner or later, there will be another influenza epidemic, which may claim a higher toll than the pandemic of 1957, especially among vulnerable persons. It is questionable to what extent a vaccine, even if available in time, will ward off the virus involved, and all other possible means to reduce the grave features of the clinical illness deserve serious consideration.

Besides the immediate dangers from influenzal pneumonia, this insult to the lungs often marks the beginning or progression of chronic pulmonary diseases. Therefore, prevention rather than treatment of secondary bacterial complications should be the ultimate goal.

#### SUMMARY AND CONCLUSIONS

Persons with chronic respiratory ailments accounted for a high percentage of the deaths and the serious illnesses during and after the pandemic of Asian influenza. Possibly to prevent severe or protracted sickness in these vulnerable patients, 75 children and 21 adults with chronic bronchitis or infectious asthma received chemoprophylaxis for influenza-like illnesses between September, 1957, and March, 1958. Penicillin, tetracycline, and, in some instances, streptomycin and chloramphenicol were the antibiotics used alone or in combinations. Thirty-six patients with asthma received anti-inflammatory steroids in addition to antimicrobial agents.

During the seven month observation period, only 1 of the antibiotically treated adult patients with influenza developed pneumonia, and 3 others had a protracted flare-up of their chronic bronchitis. Three children had a transient otitis media catarrhalis. A comparison between the school absences of children in this study and those of other susceptible children treated with conventional methods disclosed that during and after the epidemic, consistent chemotherapy greatly reduced disabling sickness.

Severe complications from prophylactic antibiotic treatment did not occur; there was neither evidence of superinfection nor an increase of *Staphylococcus* carriers as sequelae to early chemotherapy in influenza.

The results of this study suggest that chemoprophylaxis is definitely indicated in influenza-like illnesses for persons with pre-existing respiratory ailments. It also appears that there is a need for extensive investigations on the possible prevention of complications from viral respiratory infections in general.

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## BIBLIOGRAPHY

1. MORGAN, D. M., AND PICKUP, J. D.: Thirteen cases of influenzal bronchopneumonia in children, *Brit. M. J.* 1:263, 1958.
2. GILES, C., AND SHUTTLEWORTH, E. M.: Post-mortem findings in 46 influenza deaths, *Lancet* 2:1224, 1957.
3. JAMIESON, W. M.; GREEN, D. M.; DUNBAR, J. M., ET AL: Some aspects of the recent epidemic of influenza in Dundee, *Brit. M. J.* 1:908, 1958.
4. WALKER, W. C.; DOUGLAS, A. C.; LECKIE, W. J.; PINES, A., AND GRANT, J. W.: Respiratory complications of influenza, *Lancet* 1:449, 1958.
5. HERRMANN, R. E.; OGURA, G. I.; JOHNSON, E. S.; TOLL, H. W., AND WHITE, W. C.: Respiratory deaths associated with Asian influenza epidemic. Report of twenty-three cases, *J.A.M.A.* 166:467, 1958.
6. BUECHNER, H. A.; THOMPSON, J., AND MOSBY, W. H.: Fulminating post-influenzal pneumonias, *J. Louisiana M. Soc.* 110:184, 1958.
7. ANDERSON, R. J.: Influenza: 1957-1958 pandemic, *GP* 17:110, 1958.
8. PODOVIN, R. L., AND FELTON, W. L.: The clinical picture of Far East influenza occurring at the Fourth National Boy Scout Jamboree: report of 616 cases, *New England J. Med.* 258:778, 1958.
9. FINKE, W.: Prevention of chronic pulmonary disease following epidemic respiratory infection. A postwar problem, *Am. Pract.* 2:457, 1948.
10. FINKE, W.: Prospects for prevention of chronic bronchitis and bronchiectasis, *J. Pediat.* 33:29, 1948.
11. FINKE, W.: Public health aspects and prevention of bronchopulmonary disease, *J.A.M.A.* 151:105, 1953.
12. FINKE, W.: Long-term antibiotic therapy in chronic bronchitis and infectious asthma: control and prevention of bronchopulmonary disease, *Antib. & Chemo.* 4:319, 1954.
13. FINKE, W.: *Bronchiectasis. Current Therapy 1955*, Philadelphia and London, W. B. Saunders Company, 1956, p. 67.
14. FINKE, W.: Penicillin V in the prophylaxis of disabling respiratory infections in susceptible children. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 287.
15. FINKE, W.: Penicillin V, tetracycline, and prednisone in prolonged antibiotic-steroid treatment of infectious asthma in children. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 431.
16. ANON.: Special committee on influenza: Antibiotics and influenza, *J.A.M.A.* 165:58, 1957.
17. BARACH, A. L.; BICKERMAN, H. A., AND BECK, G. J.: Antibiotic therapy in infections of the respiratory tract, *A.M.A. Arch. Int. Med.* 90:808, 1952.
18. DOUGLAS, A. C.; SOMNER, A. R.; MARKS, B. L., AND GRANT, I. W.: Effect of antibiotics on purulent sputum in chronic bronchitis and bronchiectasis, *Lancet* 2:214, 1957.
19. CHAPPLE, P. A. L.; FRANKLIN, L. M.; PAULET, J. D.; TUCKMAN, E.; WOODALL, J. T.; TOMLINSON, A. J. H., AND McDONALD, J. C.: Treatment of acute sore throat in general practice: therapeutic trial, with observations on symptoms and bacteriology, *Brit. M. J.* 1:705, 1956.
20. BURN, J. J.; CURWEN, M. P.; HUNTSMAN, R. G., AND SHOOTER, R. A.: A trial of penicillin V: response of penicillin-resistant staphylococcal infections to penicillin, *Brit. M. J.* 2:193, 1957.
21. MORRISON, B.; BASS, D.; DAVIS, J. A.; HOBSON, D.; MADSEN, T. I., AND MASTERS, P. L.: Acute lower-respiratory infections in childhood, *Lancet* 2:1077, 1957.
22. ELMES, P. C.; FLETCHER, C. M., AND DUTTON, A. A.: Prophylactic use of oxytetracycline for exacerbations of chronic bronchitis, *Brit. M. J.* 2:1272, 1957.
23. EDWARDS, G.; BUCKLEY, A. R.; FEAR, E. C.; WILLIAMSON, G. M., AND ZINNEBANN, K.: Adult chronic bronchitis—the infective factor and its treatment, *Brit. M. J.* 2:259, 1957.
24. FINKE, W.: Aerosol therapy in long-term antibiotic treatment of respiratory infections, *Am. Pract. & Digest Treat.* 5:150, 1954.
25. ANON.: A report by a Subcommittee of the Antibiotics Clinical Trials (Non-tuberculous) Committee of the Medical Research Council: Prolonged antibiotic treatment of severe bronchiectasis, *Brit. M. J.* 2:255, 1957.
26. PARMELEE, A. H., AND SCHIMMEL, B. F.: Absence from school—its relationship to illness in the community, *California Med.* 88:144, 1958.
27. WILLARD, C. Y., AND HANSEN, A. E.: Influence of previous medication of antimicrobial agents on the nasopharyngeal flora of children. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 365.
28. FORKNER, C. E.: The common cold and its complications, *New York Med.* 12:58, 1956.

29. OSWALD, N.: Advances in the treatment of respiratory disorders, *Practitioner* 179:392, 1957.
30. WEINSTEIN, L., AND CHANG, T-W.: The diagnosis of virus infection, *M. Clin. North America* 41:1391, 1957.
31. ANON.: A report by the Public Health Laboratory Service based on records from hospital and public health laboratories: Death from Asian influenza, 1957, *Brit. M. J.* 1:915, 1958.
32. FINLAND, M.: Antimicrobial treatment for viral and related infections. II. Antibiotic treatment of acute respiratory infections and influenza, *New England J. Med.* 247:557, 1952.
33. KARELITZ, S.; CHANG, C. C., AND ZACHARY, E. M.: The prophylaxis and treatment of bacterial complications of measles with benzethacil and aqueous procaine penicillin G, *J. Pediat.* 44:357, 1954.
34. BARNEY, W. H.: The use of a broad-spectrum antibiotic (oxytetracycline) in the treatment of upper respiratory infections. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 895.
35. FLORENTIN, A. A., AND SISON, A. B.: The role of antibiotics in Asian influenza. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 941.
36. BURKE, J. B.: Prophylactic sulphamidine in children subject to recurrent infections of the upper respiratory tract, *Brit. M. J.* 1:538, 1956.
37. RITCHIE, J. M.: Antibiotics in small doses for the common cold, *Lancet* 1:618, 1958.
38. FRY, J.: Influenza A (Asian) 1957. Clinical and epidemiological features in a general practice, *Brit. M. J.* 1:259, 1958.
39. GREENE, L. S., AND HAIR, T. E.: Clinical experience with 682 cases of Asian influenza, *U. S. Armed Forces M. J.* 9:385, 1958.
40. MCVAY, L. V., AND SPRUNT, D. H.: Antibiotic prophylaxis in chronic respiratory diseases, *A.M.A. Arch. Int. Med.* 92:833, 1953.
41. GRATER, W. C.: Prophylaxis of recurring infection in children with asthma by the use of tetracycline, *Am. Pract. & Digest Treat.* 9:249, 1958.
42. McDERMOTT, W.: Problem of staphylococcal infection, *Ann. New York Acad. Sc.* 65:58, 1956.
43. WAISBREN, B. A.: The sensitivity of staphylococci to antibiotics. A five-year study of sensitivity, usage, and cross resistance in a general hospital, *A.M.A. Arch. Int. Med.* 101:397, 1958.
44. DINEEN, P., AND PEARCE, C.: A ten-year study on wound infections, *Surg., Gynec. & Obst.* 106:453, 1958.
45. GRIFFITH, R. S.; BONIECE, W. S.; MCGUIRE, J. M.; WOLFE, R. N.; JOINER, M.; WICK, W. E., AND HOLMES, D. H.: Biological and antibiotic sensitivity studies on "street staphylococci." *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 370.

# Spiramycin in the Treatment of Hospitalized Patients and in Male Patients with Acute Gonorrheal Urethritis

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Since the first report on spiramycin by Pinnert-Sindico and co-workers<sup>1</sup> at the Second Annual Symposium on Antibiotics in 1954, several investigators have studied this new antibiotic in laboratory and clinical investigations. Pinnert-Sindico's group described spiramycin as consisting of three active components isolated (along with congocidin) from *Streptomyces ambofaciens* and with chemical and physical properties similar to those of erythromycin and carbomycin. Clinically, they suggested that dosages of spiramycin should be higher than for erythromycin, and they gave up to 4 Gm./day without untoward effects. Further reports on spiramycin were presented at the Third Annual Symposium on Antibiotics in 1955 by Ravina et al<sup>2</sup> and Lepper et al.<sup>3</sup> Ravina and his group pointed out that the antibacterial spectrum for spiramycin covered mainly gram-positive organisms and *Neisseria*, but satisfactory bacteriostatic activity was found on *Staphylococcus aureus* or *Staphylococcus albus*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Diplococcus pneumoniae*, on the *Neisseria* group (*meningitidis*, *gonorrhoeae*, *catarrhalis*), *Corynebacterium diphtheriae*, and some anaerobes. All strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus* were found totally resistant. Spiramycin was more active in vivo than could be expected from in vitro tests, and its activity was equal to that of erythromycin. Using dosages of 3 Gm./day, given in three to four oral doses, Ravina et al found spiramycin especially effective in pleuropulmonary infections, septicemia and miscellaneous wound infections, and furunculosis, and its side effects were "innocuous." Lepper and his group<sup>3</sup> used spiramycin in 124 patients, with adults receiving a maximum of 3 Gm. daily and children receiving from 75 to 100 mg./Kg./24 hours. Serum concentration assays were low, indicating either poor absorption or rapid destruction of spiramycin. During a six month study while spiramycin was being used on patients and hospital personnel, they noted an increasing trend in the development of spiramycin-resistant strains of *Micrococcus pyogenes*. These workers also found some cross resistance with erythromycin. In a later report, Lepper et al<sup>4</sup> found that the use of novobiocin-spiramycin combinations in the treatment of infections due to "hospital" staphylococci slowed (by two months) but did not prevent the accumulation of resistant strains. However, these workers referred to their previous report,<sup>3</sup> where it was shown that the rate of accumulation of resistant strains of micrococci was more rapid when spiramycin was used alone. Lepper<sup>5</sup> has summarized his evaluation of spiramycin in a recent report.

In 1956, Hudson and his associates<sup>6</sup> reported that spiramycin was quite effective in vitro against pneumococci and fairly effective in inhibiting beta-hemolytic streptococci, nonhemolytic streptococci, and various strains of staphylococci. While they found that susceptible organisms were less sensitive to spiramycin than to erythromycin or penicillin, they showed that many penicillin- or erythromycin-resistant strains of staphylococci were sensitive to spiramycin. Blood level studies done after oral administration of 4 Gm. daily of spiramycin or 2 Gm.

daily of erythromycin showed comparable blood levels (1 to 7  $\mu\text{g.}/\text{ml.}$ ). The 29 patients with bacterial pneumonia given oral doses of 4 Gm. daily of spiramycin responded satisfactorily with a minimum of untoward actions. Willcox<sup>7</sup> gave 10 Gm. of spiramycin over a five day period to 77 patients with nongonococcal urethritis, with 16 (20.8 per cent) treatment failures, including possible reinfections, observed during a three month follow-up. Side effects occurred in 15 patients, with 14 complaining of looseness of stools; 1, dizziness; 1, bitter taste; 1, indigestion; 1, nausea and a rash; 1, abdominal pains; and 3, rectal soreness or pruritus.

In May, 1958, we started the use of spiramycin for the treatment of various types of infections encountered in the hospital. The present report deals with the use of spiramycin in the treatment of 61 hospitalized patients, 1 clinic patient, and 27 men with acute gonorrheal urethritis seen at the Portland Public Health Clinic.

#### PATIENTS, MATERIALS, AND METHODS

*Hospitalized Patients.* Fifty-six patients including 1 outpatient were treated in the Multnomah County Hospital and 6 private patients were seen at St. Vincent's Hospital. All were adults with the exception of 3 girls aged 15, 15, and 16 years, respectively. In most cases, specimens for bacteriological diagnoses, urinalysis, and white blood cell counts and differentials were obtained before, during, or after spiramycin therapy. It was possible to obtain bacteriological cultures for diagnosis before spiramycin treatment in 50 patients. No specimens were taken in 10 patients, and in 2 patients the specimens were misplaced. Nine patients had two different infectious conditions, such as furunculosis and exacerbation of chronic pyelonephritis or a wound abscess and bronchopneumonia. Using "home-made" discs, it was possible to test the sensitivity to spiramycin of the organisms found on culture in only 8 patients. In 7 of these 8 patients with nonhemolytic staphylococci, coagulase-positive, this organism was sensitive to spiramycin, while *Ps. aeruginosa* in 2 patients was insensitive. Table I shows the diagnoses and the supporting bacteriological diagnosis, when done, for the hospitalized patients.

*Dosage.* Spiramycin was available as the 250 mg. tablet. While the optimal daily dosage of spiramycin has been stated to be from 4 to 6 Gm. for the treatment of moderately severe infections, we used a dosage of 2 Gm./day in the majority of patients. No limitation was placed on the duration of treatment.

*Patients with Acute Gonorrheal Urethritis.* Twenty-seven adult men with acute gonorrheal urethritis diagnosed by smear and culture were treated with spiramycin. Based on our previous experience<sup>9</sup> and the results of others<sup>8</sup> who used tetracycline, we decided to employ a single dose of 2 Gm. of spiramycin for treatment in these patients. White blood cell counts and differentials were done before treatment and on return of the first 12 patients seen.

#### RESULTS

The response to spiramycin therapy has been classified as "good" when there was prompt and definite symptomatic improvement with temperature drop, decreased leukocytosis, clearing of the pyuria, and/or rapid wound healing and decrease in drainage from an open wound or sinus tract. An excellent response was reported by the physician directly in charge of several patients when recovery was equal to or better than could be expected with the use of tetracycline; however, we have listed these cases in the "good" category. The classification of "satisfactory"

has been used when the response was not outstanding nor as good as might have been obtained had other antibiotics or antibiotic combinations been employed, or when, in spite of good subjective and symptomatic improvement on the part of the patient, the infection was slow to heal and the temperature, leukocytosis, or pyuria was slow to return to normal. The designation of "fair" means that the patient's response was not satisfactory under the conditions of the trial but that some beneficial effect was noted.

Table II shows that staphylococci were found prior to spiramycin treatment in 23 patients. In 3 other patients (table III) staphylococci were associated with other organisms in the culture. In another patient the cause of the infection was originally due to hemolytic staphylococci, coagulase positive, but, after treatment with peni-

TABLE I  
*Diagnoses of Infections in 62 Hospitalized Patients Treated with Spiramycin*

Diagnosis	No. of cases	Bacteriological diagnosis	
		Organism	No. cases
Furunculosis	8	Nonhemolytic staphylococci, coagulase-positive	4
		Hemolytic staphylococci, coagulase-positive	3
		No culture	1
Soft-tissue, sinus tract infections	17	Nonhemolytic staphylococci, coagulase-positive	2
		Hemolytic staphylococci, coagulase-negative	1
		Hemolytic staphylococci, coagulase-positive	7
		Mixed enteric bacteria ( <i>E. coli</i> , <i>Ps. aeruginosa</i> , <i>A. aerogenes</i> , <i>Proteus</i> , and <i>paracolon</i> )	3
		Beta streptococci	1
		No cultures	3
Urinary Infections			
Pyelonephritis	10	Hemolytic staphylococci, coagulase-negative, and beta streptococci	1
		<i>E. coli</i>	2
		Hemolytic <i>E. coli</i>	1
		Hemolytic <i>E. coli</i> and <i>A. aerogenes</i>	1
		<i>A. aerogenes</i> and gamma streptococci	1
		<i>Ps. aeruginosa</i>	2
		<i>Proteus</i> , <i>Streptococcus faecalis</i>	1
		No culture	1
Cystitis	7	Hemolytic staphylococci, coagulase-positive ( <i>Proteus</i> in 1 culture)	2
		Hemolytic staphylococci, coagulase-negative	1
		<i>Ps. aeruginosa</i> , <i>E. coli</i> ( <i>Proteus</i> in 1 culture)	2
		<i>A. aerogenes</i> and <i>Bacillus subtilis</i>	1
		<i>E. coli</i> and gamma streptococci	1
Pulmonary Infections			
Lobar pneumonia	2	<i>Neisseria pharyngis</i>	1
		No culture	1
Bronchopneumonia	8	Alpha streptococci (1 culture <i>N. pharyngis</i> )	2
		Alpha hemolytic streptococci (1 culture <i>paracolon</i> )	2
		Alpha and beta streptococci	1
		<i>N. meningitidis</i> , alpha and gamma streptococci	1
		No cultures	2
Pneumonitis	6	Alpha, beta, and gamma streptococci	1
		Alpha, beta, and gamma streptococci and staphylococci	1
		Alpha streptococci and <i>N. pharyngis</i>	1
		Nonhemolytic staphylococci, coagulase-negative	1
		No culture	2
Empyema	1	Nonhemolytic staphylococci, coagulase-negative	1
Pelvic infections	3	No cultures	3
Total	62		

TABLE II  
*Treatment of Staphylococcic Infections with Spiramycin*

Diagnosis and bacteriological findings	Daily dose, Gm.	Treatment, days	Clinical response			Bacteriological follow-up	Remarks
			Good	Satis- factory	Fair		
Furunculosis							
Nonhemolytic, coagulase positive	1	13	1			No growth after 13 days treatment	
	2	7; 10	2			Not done	1 patient treated with spiramycin for 7 days also received 600,000 units procaine penicillin intra- muscularly daily for syphilis; 1 patient had "heartburn" first 4 days of treat- ment
Nonhemolytic, coag- ulase positive, and hemolytic, coagulase negative	4	7		1		Not done	
Hemolytic, coagulase positive	2	2½; 12; 12	3			Nonhemolytic, coag- ulase positive on fourth day of treat- ment in 1 patient treated 12 days	
Soft-tissue, Sinus Tract Infections							
Hemolytic, coagulase positive (3 cultures sensitive to spiramycin)	2	11; 12 14; 24	2	2		Patient treated 24 days had sterile cul- ture on fifteenth day	
Hemolytic, coagulase positive (1 insensitive to spiramycin; 1 with <i>Pseudomonas</i> , 1 with <i>E. coli</i> and paracolon)	1	4; 6; 18	2	1		Patient treated 18 days had sterile cul- ture 1 week after treatment	
Nonhemolytic, coagulase positive	2	7	1			Not checked	Dosage inade- quate
	1	16			1	Hemolytic, coagulase positive, on twelfth day	
Hemolytic, coagulase negative	1	6			1	Nonhemolytic staph- ylococci, coagulase positive on sixth day of treatment	Chloramphenicol continued while spiramycin given
Pyocystitis and Pyelitis (Urine Cultures)							
Hemolytic, coagulase positive	2	16		1		Culture plate over- grown with <i>Proteus</i> and <i>E. coli</i> on eighth day of treatment; on sixteenth day, non- hemolytic staphylo- cocci and <i>Proteus</i>	
Hemolytic, coagulase positive	2	5		1		Not done	

*Table II Continued on Page 192*

TABLE II (Continued)  
Treatment of Staphylococcal Infections with Spiramycin

Diagnosis and bacteriological findings	Daily dose, Gm.	Treatment, days	Clinical response			Bacteriological follow-up	Remarks
			Good	Satis- factory	Fair		
Hemolytic, coagulase negative (also beta streptococci in 1 patient)	2	7		1		Not done	
	4	2		1		Not done	
	6	4					Dosage increased from 4 Gm./24 hours to 6 Gm./ 24 hours
Pulmonary Infections							
Nonhemolytic, coagulase negative (empyema fluid)	4	7		1		Not done	Also received 4 injections of 200,000 units penicillin into pleural space on alternate days
(Blood culture)	4	11	1			Not done	Had pneumonitis due to leukemic infiltrates in lung; spiramycin stopped on eleventh day be- cause of thrush infection.

cillin and chloramphenicol, only *A. aerogenes*, *Ps. aeruginosa*, and paracolon were found in the pretreatment culture of the material taken from the ankle wound (table IV). A dosage of only 1 Gm./day of spiramycin was used in 1 patient with furunculosis and in 5 with soft-tissue infections for an average of 10½ days (range, 4 to 16 days) of treatment, with the response good in 3 patients and fair in 3. Follow-up cultures were reported as sterile for 2 patients, and in 1 the flora changed from hemolytic to nonhemolytic staphylococci. Ten patients with soft-tissue infections received 2 Gm./day for an average of 11 days. In 7 the response was good, and in 3, fair. One patient had "heartburn" for the first four days he was given spiramycin and another, who was receiving chloramphenicol at the same time, had urticaria for one day on the second day of spiramycin treatment. Although 3 patients with urinary infections due to staphylococci received a dosage of 2 Gm./day for periods of 5, 7, and 16 days, respectively, and another was given 4 Gm./day for two days and then 6 Gm./day for four days, the response to spiramycin was evaluated as only satisfactory. In spite of prompt subjective improvement in these patients, the temperature did not drop promptly and the pyuria was slow in clearing.

A good recovery was noted in a woman, aged 22, who had parotitis and pneumonitis due to leukemic infiltrates, with a blood culture positive for nonhemolytic staphylococci. In addition to roentgen-ray therapy and 6-mercaptopurine, she had been previously treated with penicillin, erythromycin, tetracycline, and chloramphenicol with but little benefit. Spiramycin, 4 Gm./day, controlled the fever for a 10 day period with only two spikes to 103 F. during the first five days of treatment. Unfortunately, after 11 days, spiramycin was stopped because of a severe thrush infection of the mouth. Although an effort was made to obtain follow-up bacteriological cultures during or at the end of treatment in all patients, this was

possible in only 7 in this group. In 2 patients the staphylococci appeared resistant to spiramycin: 1 patient, treated with 2 Gm./day for a period of 16 days for cystitis, with hemolytic staphylococci and *Proteus* found in the urine, on follow-up showed a culture plate overgrown with *Proteus* and *E. coli* on the eighth day of treatment, while on the last and sixteenth treatment days, nonhemolytic staphylococci and *Proteus* were present; the other patient, treated for perianal furunculosis with 2 Gm./day for 12 days, showed hemolytic staphylococci, coagulase positive, on the original culture, which changed to nonhemolytic staphylococci, coagulase positive, on the fourth day of spiramycin therapy. Unfortunately, in this case (as well as several others), it was impossible to obtain further specimens for culture.

Two of the 17 patients with pulmonary infections had lobar pneumonia, 8 had bronchopneumonia, 4 pneumonitis, and 1 a severe upper respiratory infection. The patient with empyema and 1 with leukemic infiltrates had staphylococcic infections (table II). Sputum or throat cultures were obtained from 5 patients who had bronchopneumonia, 2 who had pneumonitis, and 1 who had chronic bronchitis

TABLE III  
*Treatment of Streptococcic Infections with Spiramycin*

Diagnosis and bacteriological findings	Daily dose, Gm.	Treatment, days	Clinical response			Bacteriological follow-up	Remarks
			Good	Satisfactory	Fair		
Upper Respiratory Infection							
Alpha, beta, and/or gamma streptococci (1 culture with staphylococci)	2	5; 5	1	1			2 patients with pneumonitis showed good symptomatic improvement
	1	4	1				
Alpha streptococci (1 culture also hemolytic streptococci and paracolon; 2 cultures with <i>N. pharyngis</i> )	2	8; 8½; 8½		3		1 patient (8½ days' treatment) with hemolytic streptococci and paracolon showed only <i>N. pharyngis</i> on third day	3 patients showed symptomatic improvement in 5 days
						1 patient (8½ days' treatment) with <i>N. pharyngis</i> showed pneumococci predominating on third day of treatment	
Alpha streptococci	2	7	1				Bronchopneumonia cleared in 4 days
Alpha-hemolytic streptococci	2	5	1				Prompt resolution of bronchopneumonia
Soft-tissue Infection							
Beta streptococci	2	2	1				
Urinary Tract Infection							
Gamma streptococci and <i>E. coli</i>	1	4½	1				Prompt clearing of pyuria
<i>Str. faecalis</i> and <i>E. coli</i>	2	3½		1			Increasing dosages of spiramycin cleared pyuria
	4	1					
	6	2					

(table III). In only a few patients could the organisms isolated be considered as pathognomonic. Dosages of 2 Gm./day were used for periods of 5 to 8½ days with good symptomatic improvement in 3 patients, while the response was somewhat slower but satisfactory in 4 patients. Attention is called to the woman, aged 23, who received spiramycin in 1 Gm./day dosage for four days, because of the occurrence of leukopenia during therapy. One month previously she had delivered a normal infant, and she entered the hospital with a jaw infection after exodontia, bronchopneumonia, septic sore throat, and acute endometritis. While she made a prompt recovery, the white blood cell count of 4850 cells/cu. mm. with 62 per cent polymorphonuclear cells, noted a day before spiramycin was started, dropped to 2800 cells/cu. mm. with 62 per cent polymorphonuclear cells on the second day of treatment and on the third day to 1920 cells/cu. mm. and 18 per cent polymorphonuclear cells. One day after completion of spiramycin therapy, the leukocytes were 3075 cells/cu. mm. with 32 per cent polymorphonuclears and 6 per cent eosinophils, while 11 days after treatment, the count was 7650 cells/cu. mm. and

TABLE IV

Treatment of Infections with Spiramycin When Enteric Bacteria Found in Culture Specimen

Diagnosis and bacteriological findings	Daily dose, Gm.	Treatment, days	Clinical response			Bacteriological follow-up	Remarks
			Good	Satisfactory	Fair		
Urinary Infections							
<i>A. aerogenes</i> (1 culture also <i>B. subtilis</i> ; 1 culture with gamma streptococci)	2	7; 11	1	1		Not done	
<i>Ps. aeruginosa</i> (2 cultures with <i>E. coli</i> and <i>Proteus</i> )	2	4½; 5; 6½; 7	3	1		1 patient (6½ days' treatment) still showed <i>Ps. aeruginosa</i> 1 week after treatment	
<i>E. coli</i>	2	5; 6	2				
Hemolytic <i>E. coli</i>  (1 culture with <i>A. aerogenes</i> )	4	8				Fail- 5 days after treatment <i>E. coli</i> present	No response in any symptoms
	2	7	1			On sixth day of treatment hemolytic <i>E. coli</i> and <i>A. aerogenes</i> present	
Soft-tissue Infections							
<i>A. aerogenes</i> , <i>Ps. aeruginosa</i> , and <i>E. coli</i>	4	21		1		All organisms present in wound culture on tenth day of treatment	Prior to spiramycin therapy given 600,000 units procaine penicillin twice daily for 6 days for lobar pneumonia
<i>A. aerogenes</i> , <i>Ps. aeruginosa</i> , and paracolon	2	17			1	After 17 days' treatment culture showed nonhemolytic staphylococci and <i>A. aerogenes</i>	Infection originally caused by hemolytic <i>Staphylococcus</i> ; treated with penicillin and chloramphenicol prior to use of spiramycin

TABLE V  
*Spiramycin Treatment in Patients with or without Pretreatment Cultures*

Diagnosis and bacteriological findings	Daily dose, Gm.	Treatment, days	Clinical response		Remarks
			Good	Satisfactory Fair	
Lobar pneumonia	2	7; 10	2		Prompt symptomatic improvement and resolution within 5 days
Bronchopneumonia ( <i>N. meningitidis</i> and alpha and gamma streptococci in sputum of 1 patient; <i>N. pharyngis</i> in throat culture 1 patient)	2 1	7; 7; 7 5	3 1		All patients showed prompt recovery
Pneumonitis	2	3		1	Slow resolution but no follow-up as patient left hospital
Sinusitis, acute	2	6	1		
Cellulitis, postoperative, wound incision (urine culture showed <i>Pseudomonas</i> insensitive to all antibiotics used)	2	6		1	
Ulceration, scrotum	2	7½	1		
Thrombophlebitis	1	2		1	
Pyelonephritis	2	4	1		
Endometritis	2	4	1		
Abscess, tubo-ovarian	2	3	1		
Salpingitis	2	8		1	

46 per cent polymorphonuclears. It was felt that this transient leukopenia was more likely due to a concomitant virus infection than to a spiramycin toxic effect.

Only 1 of the 14 patients with soft-tissue infections from whom specimens for culture were obtained harbored streptococci. This patient had been bitten on the dorsum of the hand by a prostitute and three days later developed chills and fever along with an extensive cellulitis of the hand and a purulent discharge from the wound. Prompt subsidence of the infection occurred when he was treated with 2 Gm./day of spiramycin for two days. A satisfactory response was reported for the 2 patients with urinary tract infection due to streptococci.

Ten patients with urinary tract infections harbored enteric bacteria (table IV). In spite of the demonstrated ineffectiveness of spiramycin against organisms such as *Aerobacter*, *Pseudomonas*, *Proteus*, and *E. coli*,<sup>2</sup> 7 of these patients showed good symptomatic improvement within a few days and were treated an average of only six days. Follow-up urine cultures in 2 patients showed the presence of the same organism originally found. The patient considered a failure experienced no subjective or symptomatic improvement; in fact, the fever, leukocytosis, and pyuria worsened. This patient was given 4 Gm./day of spiramycin for eight days for pyelonephritis because she entered the hospital for control of diabetes mellitus. Enteric bacteria were found in the urine of 1 patient treated for furunculosis and another who had a postoperative wound incision cellulitis; no specimens of pus for culture were obtained from either of these patients. Six patients with soft-tissue infections were found to have enteric bacteria in the cultures made from pus; in 4

patients, other organisms such as staphylococci or streptococci were also reported and considered as the offending agent, while the other 2 patients showed only enteric bacteria on culture.

Of interest are the bacteriological findings for the patient who received spiramycin for 17 days for treatment of a chronic abscess about the ankle. In March, 1958, this 35 year old man sprained the left ankle severely and eight days later a large abscess formed about the ankle. After incision and drainage, he was treated with procaine penicillin for six days and then chloramphenicol for the next 15 days. Without complete healing of the ulcer, the leg was placed in a cast. Several concentration tests and cultures for *Mycobacterium tuberculosis* were negative and several roentgenograms showed no osteomyelitis. A month after antibiotic treatment, culture of the material from the draining ulcer revealed hemolytic staphylococci, coagulase positive, as well as nonhemolytic staphylococci, coagulase positive. When spiramycin was started three months later, *Aerobacter*, *Pseudomonas*, and paracolon were found on culture; after 17 days of treatment, the organisms reported were nonhemolytic staphylococci, coagulase positive, and *Aerobacter*.

Table V completes the record of spiramycin treatment in the hospitalized patients. Ten patients with various types of infection received dosages of 2 Gm./day for periods of from 3 to 10 days with a good response, as did 1 patient treated with 1 Gm./day for five days. Two patients given 2 Gm./day for three and six days, respectively, were reported as having a satisfactory response. A woman with thrombophlebitis of the leg after an infected ulcer on the ankle received 1 Gm./day for only two days; because of dramatic improvement in her condition, she left the hospital in order to care for her children before completing treatment and, consequently, her response has been classified as satisfactory. Another patient with bronchopneumonia showed a good response after treatment with 1 Gm./day for five days. A patient with pneumonitis who was started on a 2 Gm./day dosage of spiramycin left the hospital after three days' treatment because she felt subjectively improved; however, because she received insufficient treatment she has been placed in the "fair" response category.

*Patients with Acute Gonorrheal Urethritis.* A single oral dose of 2 Gm. of spiramycin was administered to 26 patients and a dosage of 2 Gm./day to 1 patient for three days in males with acute gonorrheal urethritis. Of the 19 reporting back for follow-up smear and culture, 16 (84.2 per cent) were cured while 3 were considered failures. No further follow-up was done after the patient reported back once, and since none of these patients later returned to the clinic with the same complaint, it is assumed that cure was obtained. Mild side effects (loose stools, nausea, pruritus ani) were reported for only 3 (15.8 per cent) patients. White blood cell counts and differentials, done on 12 patients before and after spiramycin therapy, were within normal limits.

#### COMMENT

The customary procedure followed in this hospital and others in this area is first to use procaine penicillin and streptomycin intramuscularly for the treatment of all serious infectious conditions. Then, if the infection does not show improvement or the culture sensitivity tests so indicate, another antibiotic is tried. Usually, dependence is placed on tetracycline, although at present chloramphenicol is frequently used, while novobiocin is occasionally employed and erythromycin or oleandomycin rarely. Thus, in the present study our clinical impression of the

effectiveness of spiramycin has been influenced somewhat by our experience with tetracycline and chloramphenicol and our previous use of novobiocin.<sup>10-12</sup> In some instances our evaluation of the response to spiramycin as "good" may have been too conservative, since the attending staff physicians considered the therapeutic results as excellent in several patients. However, in the complete evaluation of the patient's over-all response to spiramycin, the lack of prompt symptomatic improvement or return of the laboratory or roentgenological findings to within normal limits influenced our decision in this matter.

When it is considered that relatively low dosages (1 Gm./day in 7 patients and 2 Gm./day in 47 patients) were employed in the majority of patients, with a good or satisfactory response obtained in 54 (86 per cent) of the patients, the use of spiramycin appears quite promising. Even in those patients with urinary infections and the presence of enteric bacteria known to be insensitive to spiramycin, the symptomatic improvement and the decrease in abnormal urinary findings occurred as promptly and as completely had tetracycline or novobiocin been used. While there was some indication of the development of resistance to spiramycin in 2 of our patients, unfortunately too few follow-up cultures were obtained in this study to allow making any conclusions relative to this important problem with respect to the status of spiramycin. In spite of our favorable results with spiramycin when used in dosages of 1 Gm./day to 2 Gm./day, we are of the opinion that the preferred dosage is 4 Gm./day for the treatment of the majority of moderately severe infections encountered in the hospital. In view of the remarkable lack of toxic effects seen when spiramycin is used, there should be no difficulty experienced with the 4 Gm./day dosage, and its use would hasten recovery and shorten the duration of treatment.

Our results in treating male patients with acute gonorrheal urethritis with single doses of 2 Gm. of spiramycin are interesting. While the cure rate of 84.2 per cent obtained in 19 patients who returned for follow-up may not be as dramatic as the results obtained by Buckinger and Hookings<sup>8</sup> with tetracycline, nevertheless this result indicates the efficacy of spiramycin in this disease.

There is no doubt that spiramycin is a useful drug and gratifyingly "innocuous" with respect to the appearance of untoward effects. It is at least equal in efficacy and spectrum of activity to some of the orally administered antibiotics now available and, in our opinion, at present superior to others. It remains to be determined whether or not its usefulness will be limited by the rapid development of resistant strains of micrococci after its wide use.

#### SUMMARY

1. Spiramycin has been used for the treatment of various types of infections in 62 hospitalized patients and in 19 male patients with acute gonorrheal urethritis.

2. Cultures of the sputum, urine, pus, or blood obtained for diagnosis in 50 hospitalized patients showed the predominant organism to be staphylococci in 26 patients, streptococci in 10, various enteric bacteria in 12, and mixed oral cavity flora in 2. No cultures were obtained from 12 patients.

3. Eight patients were diagnosed as having bronchopneumonia; 6, pneumonitis; 2, lobar pneumonia; 19, wound, sinus tract, or suppurative lesions; 7, furunculosis; 17, urinary infections; and 3, pelvic inflammatory disease.

4. Using dosages of 1 Gm./day for 4 to 11 days in 7 patients, 2 Gm./day for 2½ to 17 days in 47 patients, 4 Gm./day for 4 to 20 days in 6 patients, and

increasing dosages from 4 Gm./day to 6 Gm./day in 2 patients, the response to spiramycin was considered as "good" in 32 (51 per cent), "satisfactory" in 22 (35 per cent), "fair" in 7 (11 per cent), and a failure in 1 patient.

5. In 2 patients with staphylococcic infections there was some evidence of the development of resistance as indicated by study of follow-up cultures.

6. A single dose of 2 Gm. of spiramycin cured acute gonorrheal urethritis in 16 (84.2 per cent) of the 19 male patients treated.

7. There were remarkably few side effects in either the hospital-treated patients or those given single doses of spiramycin for acute gonorrheal urethritis. These effects were mostly referable to the gastrointestinal tract and were short-lasting.

#### BIBLIOGRAPHY

1. PINNERT-SINDICO, S.; NINET, L.; PREUD'HOMME, J., AND COSAR, C.: A new antibiotic—spiramycin. *In: Antibiotics Annual 1954–1955*, New York, Medical Encyclopedia, Inc., 1955, p. 724.
2. RAVINA, A.; PESTEL, M.; ELOY, P.; DUCHESNAY, G.; ALBOUY, R., AND REY, M.: A new French antibiotic: spiramycin. *In: Antibiotics Annual 1955–1956*, New York, Medical Encyclopedia, Inc., 1956, p. 223.
3. LEPPER, M. H.; SPIES, H. W.; KELLOW, W. F. T.; ROSENTHAL, I. M., AND PLAUT, S.: Spiramycin in the treatment of infection. *In: Antibiotics Annual 1955–1956*, New York, Medical Encyclopedia, Inc., 1956, p. 658.
4. LEPPER, M. H.; DOWLING, H. F.; JACKSON, G. G.; SPIES, H. W., AND MELLODY, M.: The effect of the routine use of novobiocin and spiramycin in combination on the antibiotic sensitiveness of hospital staphylococci. *In: Antibiotics Annual 1956–1957*, New York, Medical Encyclopedia, Inc., 1957, p. 640.
5. LEPPER, M. H.: Current therapeutics, CXI. Spiramycin, *Practitioner* 178:363–368, 1957.
6. HUDSON, D. G.; YOSHIHARA, G. M., AND KIRBY, W. M. M.: Spiramycin—clinical and laboratory studies, *A.M.A. Arch. Int. Med.* 97:57–61, 1956.
7. WILLCOX, R. R.: Spiramycin in the treatment of nongonococcal urethritis, *AM & CT* 4:343–346, 1957.
8. BUCKINGER, R. H., AND HOOKINGS, C. E.: Single-dose treatment of acute gonorrhea with oral tetracycline. *In: Antibiotic Annual 1954–1955*, New York, Medical Encyclopedia, Inc., 1955, p. 574.
9. DAVID, N. A.: Erythromycin, oxytetracycline and tetracycline in the treatment of gonorrheal urethritis. *In: Antibiotics Annual 1954–1955*, New York, Medical Encyclopedia, Inc., 1955, p. 699.
10. DAVID, N. A., AND BURGNER, P. R.: Clinical effectiveness and safety of novobiocin, *Antib. Med.* 2:219–229, 1956.
11. DAVID, N. A.; MCCAWLEY, E. L., AND DEBOLT, W. L.: New novobiocin preparations in hospital practice. *In: Antibiotics Annual 1956–1957*, New York, Medical Encyclopedia, Inc., 1957, p. 402.
12. DAVID, N. A., AND DAY, W. R.: Use of a new tetracycline-novobiocin combination in the treatment of various types of infections, *AM & CT* 5:128–134, 1958.

# Experience with Spiramycin, a New Antibiotic in Ophthalmic Infections

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Bacterial resistance to antibiotics, particularly the staphylococcic bacilli, has led to an increased interest in the development of newer antibiotics that could possibly replace those varieties to which bacteria become resistant.

Spiramycin is one of these newer antibiotics. It was originally isolated from a soil sample in France in 1951 and is derived from *Streptomyces ambofaciens*.

Essentially, spiramycin possesses the same antibacterial spectrum as erythromycin and oleandomycin. Clinically, spiramycin has been reported<sup>1</sup> effective in staphylococcic infection,<sup>2-4</sup> streptococcic infection,<sup>2,3</sup> enterococcic infection,<sup>2-4</sup> syphilis,<sup>2</sup> and gonorrhea,<sup>2,5,6</sup> and approximately equal in effectiveness to erythromycin, penicillin, and the tetracyclines. It is especially active on gram-positive bacteria and to a lesser degree against mycobacteria and gram-negative bacteria. Spiramycin has been found active against various staphylococcic infections (furunculosis, osteomyelitis) where the *Staphylococcus* has become resistant to other antibiotics. It is also fast-acting in gonococcic infections.<sup>7</sup> Its physicochemical and antibacterial properties are therefore similar to those of most of the antibiotics in wide usage today.

Willcox<sup>8,9</sup> found spiramycin well tolerated and satisfactory for use in non-gonococcal urethritis. According to this author, the drug compared favorably with the tetracyclines and was superior to erythromycin, streptomycin, sulfonamides, penicillin, chloramphenicol, novobiocin, or aminitizole.

Finland,<sup>10</sup> in studies on cross-resistance with the newer antibiotics that demonstrate antistaphylococcic activity, noted only a small degree of difference, percentage-wise, among all of them. Among the agents he examined were erythromycin, carbomycin, oleandomycin, streptogamin, and spiramycin. He hastened to add in his article that, "despite this cross-resistance, the clinical significance of cross-resistance of antibiotics does not necessarily have a parallel in the patient, . . ." more specifically ". . . cross-resistance *in vitro* does not necessarily indicate similar cross-resistance *in vivo* and should not discourage clinical studies with such agents."

Since most of the clinical work with spiramycin has been done parenterally or systematically, we decided to determine whether or not the drug had comparable or greater topical therapeutic potential than other antibiotics, particularly in ophthalmic infections.

## MATERIAL AND PATIENTS

We used spiramycin as an ophthalmic ointment and solution\* in a concentration of 1 per cent in 54 patients with varied ophthalmic conditions in order to determine its efficacy. It was primarily employed in infectious external ocular conditions associated with such symptoms as grittiness or sandy sensation, swelling of lids, purulent or serous discharges, photophobia, ocular injection, and pain. Among the conditions seen were chalazion, ulcerative blepharitis, hordeolum, blepharitis squamosa,

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\* Spiramycin ointment and solution were kindly supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

acute keratoconjunctivitis, angular conjunctivitis, postoperative conjunctivitis, chronic catarrhal and follicular conjunctivitis, epiphora, acute and subacute catarrhal conjunctivitis, bullous keratitis, allergic plus infectious conjunctivitis, acute meibomianitis, catarrhal limbal ulcer, and central corneal ulcer. The ointment was used in 41 patients and the solution in 13. No bacteriological examinations were made.

## RESULTS

Of the 54 patients, 47 were improved or cured. Of these, 19 were markedly improved, 26 moderately so, and only 2 slightly improved. Symptoms associated with the conditions disappeared on improvement. Five patients did not respond to spiramycin. Two patients acquired a contact dermatitis around the eyelids. Therapy was discontinued in these 2 although it was believed that sensitivity may have been due to the ointment base rather than to spiramycin. The following cases are illustrative of the effectiveness or lack of effectiveness of this new preparation.

*Case 1.* On May 22 Mrs. E. B. complained of pain in the left eye for the previous three days. Examination revealed marked keratoconjunctivitis with a circumcorneal catarrhal ulcer involving the limbus with marked bulbar injection. Spiramycin solution, 1 per cent, was prescribed to be applied locally to the left eye every two hours. On May 26 there was residual mild bulbar injection, and the ulcer was almost completely improved.

*Case 2.* On May 23 Mrs. P. M. complained of sandy or gritty sensation of both eyes with swelling of lids for 10 days. Examination revealed bilateral chronic palpebral catarrhal conjunctivitis of all four lids with papillary and granular hypertrophy and thickening of palpebral conjunctiva. Spiramycin ointment was to be applied locally twice daily. By May 27 marked objective improvement with subsidence and elimination of subjective symptoms was noted.

*Case 3.* On June 13 Mrs. A. M. complained of swelling of the right lower lid and pain with sensation of a foreign body present for two days. Examination revealed marked acute catarrhal conjunctivitis. Spiramycin ointment, 1 per cent, was ordered to be applied locally four times daily. By June 17 there was marked subjective and objective improvement. By June 24 improvement continued, and by July 1 improvement was almost complete. On July 8 she was still under treatment for recurrence of redness and the foreign body sensation, with marked acute follicular palpebral conjunctivitis of the right upper and lower lids. The condition responded favorably to local sulfonamide treatment.

*Case 4.* On May 23 Mrs. C. L. complained of purulent discharge and morning agglutination of lids associated with redness of both eyes. Examination revealed marked acute catarrhal conjunctivitis of both eyes. Spiramycin ointment, 1 per cent, applied locally four times daily was recommended. By May 27 there was marked subjective improvement. Moderate injection of palpebral conjunctiva was still present. She was told to continue spiramycin ointment twice daily. By June 3 she was completely improved.

## SUMMARY AND CONCLUSIONS

Spiramycin in 1 per cent ointment and 1 per cent solution was used for local treatment of external ocular infectious conditions in 54 patients. Varying degrees of improvement were noted in 47 of the 54 patients, with more than 83 per cent demonstrating moderate to marked improvement.

Acute and chronic conjunctivitis responded well to spiramycin. The solution and ointment were nonirritating and acceptable to the patients.

It is our opinion that spiramycin is a potentially good topical ointment or solution in external eye disorders. We would conclude therefore that spiramycin could be used in the topical treatment of various ophthalmic diseases as a substitute for

the many antibiotics currently available, but is not especially superior to any of these agents nor as effective as the steroids.

#### BIBLIOGRAPHY

1. ANON.: Editorial Comments: Spiramycin, *Canad. M. A. J.* 78:713, 1958.
2. DARBON, A., AND CROSNIER, R.: Un an d'experimentation clinique de la spiramycine en pathologie infectieuse, *Presse med.* 63:681, 1955.
3. DUREL, M. P.: A new antibiotic, spiramycin, *Thérapie* 10:437, 1955.
4. RAVINA, A.; PESTEL, M.; ELOY, P.; DUCHESNAY, G.; ALBOUY, R., AND REY, M.: A new French antibiotic: spiramycin. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, p. 223.
5. SYLVESTRE, L., AND ETHIER, J.: La Rovamycine dans le traitement des urétrites gonococciques, *Union méd. du Canada* 86:657, 1957.
6. WILLCOX, R. R.: Treatment of gonorrhoea with spiramycin, *Brit. J. Ven. Dis.* 32:117, 1956.
7. PINNERT-SINDICO, S.; NINET, L.; PREUD'HOMME, J., AND COSAR, C.: A new antibiotic—spiramycin. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 724.
8. WILLCOX, R. R.: Treatment of nongonococcal urethritis with spiramycin, *Brit. J. Ven. Dis.* 32:115, 1956.
9. WILLCOX, R. R.: The treatment of non-gonococcal urethritis with spiramycin (Rovamycin), *Brit. J. Clin. Pract.* 12:7, 1958.
10. FINLAND, M.: Cross-resistance among five antibiotics, *Gior. di Microbiologia* 2:371, 1956.

# The Use of Spiramycin for Acute Gonococcal Urethritis in Men

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Pinnert-Sindico and co-workers<sup>1</sup> reported the isolation of spiramycin in 1955. These authors considered the new antibiotic similar to erythromycin. However, a somewhat higher dosage of spiramycin was required for clinical response, as compared with the therapeutic dose of erythromycin. Since then a number of papers have reported the use of spiramycin in various infectious diseases. Pinnert-Sindico stated that one day of treatment with 2 Gm. of the antibiotic was sufficient for the cure of gonococcal urethritis in men. Willcox<sup>2</sup> reported good results in controlling this infection with 4 to 12 Gm. of spiramycin administered over a period of two days. Lowering the dose produced less favorable results.

This paper presents our experience with peroral spiramycin in the treatment of 94 men with gonococcal urethritis.

## METHODS

The choice of patients, the laboratory methods employed, and the criteria of cure have been previously detailed.<sup>3</sup>

All patients presented clinical and laboratory manifestations of gonorrhea, and none had received medication for the present illness. Spiramycin, which was available in 250 mg. tablets, was administered orally in the following dose schedules: (1) 500 mg., four times daily for one day; (2) 500 mg., four times daily for two days; (3) 500 mg., four times daily for three days; and (4) 500 mg., four times daily for four days.

The patients were considered cured when the urethral discharge disappeared, and smears and cultures were negative for a minimum of four post-treatment days.

## RESULTS

Table I presents the results obtained with the various dose schedules. The 16 patients treated with a total of 8 Gm. of spiramycin all responded favorably to the drug. One patient, however, who returned for the third post-treatment culture nine days following termination of therapy, had a purulent discharge positive for gonococci. He denied sexual contact during the treatment and post-treatment period. This patient's history of four gonococcal infections within a three month period created the impression that the positive purulent discharge was due to newly acquired gonorrhea. As seen in the table, lowering the dosage reduced the number of cures. Doses below the 8 Gm. level are thus, according to the present study, inadequate for the treatment of gonorrhea.

There was one allergic reaction, consisting of flushing and pruritic urticaria, in a patient who received a total of 6 Gm. of the antibiotic. This individual, however,

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This investigation was supported, in part, by a grant from Ciba Pharmaceutical Products, Inc., Summit, N. J., and the spiramycin was furnished through the courtesy of this company.

TABLE I  
*Spiramycin in the Treatment of Gonorrhea in Men*

Total dose, Gm.	Dosage schedule, 4 times daily	No. patients treated and observed	Results	
			Cures	Failures
8	500 mg. for 4 days	16	15	1
6	500 mg. for 3 days	27	23	4
4	500 mg. for 2 days	8	6	2
2	500 mg. for 1 day	43	33	10

had previously demonstrated sensitivity to penicillin and tetracycline. In no other patient in this investigation was there any toxic or allergic manifestation.

#### DISCUSSION

Even with the cooperation of the patient, differentiation between relapse and re-infection may be a difficult task. Where the patient is uncooperative, such distinction may prove impossible and interpretation must rest on impressions.

Experience has shown that the recidivistic venereal disease patient is generally most uncooperative, and it is this patient who poses relapse-reinfection problems. The differences in the results obtained in our clinic and those reported in the literature may be due to this variable, based on interpretation of whether a post-treatment positive finding represents relapse or reinfection. Further studies are indicated to establish unequivocally the dosage level of spiramycin that will cure gonorrheal infection.

#### SUMMARY

Ninety-four patients with gonorrhea were treated under various dosage schedules with spiramycin. Eight Gm. of the antibiotic in divided doses gave 15 cures out of 16 trials. The one failure in this group is considered a probable reinfection. Lower doses of spiramycin were found to be therapeutically inadequate. One patient with a history of allergy to penicillin and tetracycline complained of flushing and pruritic urticaria.

#### BIBLIOGRAPHY

1. PINNERT-SINDICO, S.; NINET, L.; PREUD'HOMME, J., AND COSAR, C.: A new antibiotic—spiramycin. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, pp. 724-727.
2. WILLCOX, R. R.: Treatment of gonorrhea with spiramycin, *Brit. J. Ven. Dis.* 32:117, 1956.
3. MARMELL, M., AND PRIGOT, A.: Intramuscular oxytetracycline in the treatment of acute gonorrheal urethritis in the male. *Internat. Rec. Med.* 168:254, 1955.

# The Ocular Penetration and Tolerance of a New Antibiotic: Spiramycin

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In 1954 a newly discovered antibiotic, spiramycin (Rovamycine), was reported at the Second Annual Symposium on Antibiotics in Washington. Produced by the mold *Streptomyces ambofaciens* obtained in a soil sample from northern France, spiramycin was found to be identical with the Foromacidins A, B, and C derived of soil specimens from Rome and Switzerland.<sup>1,5</sup>

Spiramycin is an amorphous base slightly soluble in water and soluble in most organic solvents. The sulfate salt is soluble in water and the lower aliphatic alcohols.<sup>1</sup> Chemical tests for the identification of spiramycin have been reported by Laubie.<sup>2</sup> At room temperature the powdered drug is more soluble in phosphate buffer than in normal saline. However, with decreasing temperature solubility increased for both saline and buffer solutions.

This antibiotic has an antibacterial spectrum similar to penicillin, erythromycin, and the erythromycin-like group (oleandomycin, carbomycin) of drugs. Bacterial cross resistance can be developed between spiramycin and the erythromycin group but none has been shown toward penicillin.<sup>3,6</sup> In an excellent study of antibiotic combinations by Jones and Finland these authors point out that spiramycin or other erythromycin-like drugs combined with tetracycline seems to offer no advantage or greater antibacterial effect than when either agent is used alone.<sup>7,8</sup> The drug is mainly effective against gram-positive organisms (staphylococci, streptococci, and pneumococci) and the *Neisseria* group.<sup>1,3,4</sup> In addition certain strains of staphylococci resistant to penicillin and other antibiotics are sensitive to spiramycin.

Early clinical and laboratory trials made abroad reported the drug to be well tolerated and effective against a variety of bacterial infections and some viral pneumonias.<sup>9</sup>

Toxicity to spiramycin is of a low order mainly affecting the gastrointestinal tract. Acute oral toxicity has been produced in dogs by Boyd whose work suggests that higher oral doses might be tolerated in man than the presently suggested dosage of 3 to 4 Gm./day.<sup>10</sup>

The present study was undertaken to determine the ocular penetration and tolerance of spiramycin under normal and certain abnormal ocular circumstances.

## MATERIALS AND METHODS

Albino rabbits averaging 2 to 2.5 Kg. body weight were used in all experiments. After local application of the drug and prior to every ocular manipulation, the eye was copiously irrigated with sterile saline, and sterile tetracaine (0.5 per cent) was instilled in the lower conjunctival cul de sac. Aqueous and vitreous were aspirated by use of sterile tuberculin syringes (wetted with 1:100 heparin) and 27 and 18 gauge needles respectively.

Assays of ocular fluids or serum were run on the same day and the results read

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Ciba Pharmaceutical Products Inc. made available the spiramycin used in this study.

after 24 hours incubation at 37 C. Trypticase soy broth was utilized throughout in 1 ml. amounts in standard culture tubes. The technique employed for the assay of spiramycin in ocular fluid or serum samples was by the tube serial dilution method. Since the ocular fluids were obtainable in small amounts (0.2 ml.), the aqueous of each test animal or identical test group was pooled. In like manner, the vitreous was pooled as was also the serum. One-half ml. volumes of the pooled material for assay were placed in 1.5 ml. of broth giving a 1:4 dilution of the sample. Twofold serial dilutions were then made by serial passage of 1 ml. of the preceding dilution into 1 ml. volumes of broth, and carried from 1:4 to 1:64.

A sensitive strain of *Bacillus subtilis*, ATCC 6633 was employed in the bioassay procedure. An 18 hour agar slant culture of the organism was suspended in sterile normal saline to give an optical density in the range of 65 to 75 units on the Klett-Summerson photoelectric colorimeter using a number 54 green filter and normal saline as a blank. This bacterial suspension diluted 1:5 in saline served as the inoculum for each broth mixture by adding 0.05 ml. amounts of the suspension into each tube. By the pour plate technique, 0.05 ml. of a 1:5 Klett 70 suspension of bacteria contained roughly 60,000 viable organisms.

Spiramycin test samples for penetration or tolerance study were freshly prepared each day from the powdered drug and dissolved in phosphate buffer ( $M/20$ , pH 7.2). This material was diluted in Trypticase soy broth to make the spiramycin standard ranging arithmetically from 1 to 10  $\mu\text{g.}/\text{ml.}$  The standard was always made from the test solution of drug being studied. It was consistently found, except in two instances, that after 24 hours' incubation, 3  $\mu\text{g.}$  of spiramycin completely inhibited the growth of 0.05 ml. *B. subtilis* suspension. By this method, the first tube (i.e., 1:4 dilution) of any test fluid, would have to contain 3  $\mu\text{g.}$  or more of drug in order to be detected. Consequently spiramycin at a concentration less than 12  $\mu\text{g.}/\text{ml.}$  in the undiluted test fluids would not be detectable. Mixtures of broth and ocular fluids without added *B. subtilis* were run as controls to guard against contamination during the procedures.

## RESULTS

*Toxicity and Tolerance.* DRY POWDER. The powdered form of spiramycin when dusted on the normal cornea was moderately toxic. After two to three hours the eyes showed chemosis and moderate conjunctival hyperemia with clouding of the corneal epithelium. These findings gradually subsided and reverted to normal within 48 hours. On the abraded cornea the pure powder produced more marked and diffuse tissue damage. Corneal edema was widespread with profuse exudation. Staining of the corneal epithelium was evident even after 48 to 72 hours with persistent edema and vascular congestion of the iris.

SOLUTIONS AND OINTMENTS. Topical application of solutions were given in drop form at a rate of three drops every five minutes for one-half hour. On the normal eye spiramycin ointment in aquaphor base or solutions ranging in concentration from 0.5 to 5 per cent were nontoxic. Transient minimal hyperemia and watery discharge was apparent when a 5 per cent solution was applied to eyes with partially de-epithelialized corneas. Lower concentrations under the same abnormal conditions produced no notable abnormal changes. Ten per cent solutions made up in normal saline or buffer tended to precipitate out of solution at room temperature. It was found that their solubility increased by cooling in an ice bath. Both saline and buffer preparations in 10 per cent concentrations were irritating and toxic when applied in drop form either cold or at room temperature. The abnormal changes manifested

were profuse watery discharge of the conjunctiva, chemosis and denudation of 30 to 50 per cent of the corneal epithelium associated with congestion of the iris. One of six animal eyes showed beginning pannus formation after 36 hours.

**INTRACAMERAL AND SUBCONJUNCTIVAL INJECTIONS.** Buffer solutions (containing 500 to 10,000  $\mu\text{g.}/\text{ml.}$ ) injected beneath the superior bulbar conjunctiva in 0.5 ml. amounts were well tolerated. Larger concentrations (10,000–40,000  $\mu\text{g.}/\text{ml.}$ ) by this same route produced watery discharge, hyperemic, and chemotic changes that lasted 48 hours. No permanent sequelae were noted after one week of observation.

The tolerance to intracameral injections was more difficult to evaluate because of the trauma incident to the procedure. Several methods were utilized: (1) removal of aqueous by aspiration followed by injection of same volume of antibiotic solution through the same needle; (2) aspiration of one-half the aqueous volume with admixture of aqueous and test solution in the syringe and replacement of 0.1 ml. of the mixture; (3) injection of test solution (0.1 ml.) directly into anterior chamber without prior removal of aqueous. Animal movement, uncontrolled leakage, and occasional pricking of the iris surface or corneal endothelium by the needle occurred. In the latter instances observed changes attributable to the drug or trauma could not be separated. Nevertheless nontoxic and well-tolerated amounts in the aqueous were 250  $\mu\text{g.}$  or less. Larger quantities produced iritis, flare, persistent corneal edema and clouding of the anterior surface of the lens.

**INTRAVENOUS AND INTRAVITREAL INJECTIONS.** No ocular abnormalities were noticed with single intravenous injections containing 10,000 to 200,000  $\mu\text{g.}$  Direct injection of buffered solutions into the vitreous in 100, 250, and 500  $\mu\text{g.}$  amounts were well tolerated with no notable toxic effects. Higher quantities between 1000 and 2500  $\mu\text{g.}$  produced exudative reaction within the posterior segment of the eye with clouding of posterior lens capsule.

Table I summarizes the maximum dosage and route that was well tolerated in rabbit eyes with minimal or no reaction and without sequelae.

RESULTS OF PENETRATION STUDIES

*Topical Application on Normal Eyes.* Spiramycin in solutions of 10,000, 25,000, and 50,000  $\mu\text{g.}/\text{ml.}$  were used at a rate of three drops every five minutes for four doses. Aqueous was removed after one, two, and four hour periods from the time of initial instillation. The drug was not detected in any of the aqueous samples.

Likewise 1 and 5 per cent ointment made up in hydrophilic water miscible base showed no detectable penetration into the aqueous one, two, and four hours after application. Ten per cent solution in phosphate buffer produced 12  $\mu\text{g.}$  of spiramycin in the aqueous after 1 hour but none was detected after two or four hours.

*Topical Application on Eyes with Mechanically Abraded Corneas.* Buffer solu-

TABLE I  
*Maximal Spiramycin Dosage and Route Well Tolerated Locally by Rabbit Eyes*

Preparation	Route	Per cent or concentration
Phosphate buffer solutions	Topical	0.5–5.0*
Phosphate buffer solutions	Subconjunctival	5000 $\mu\text{g.}$ †
Phosphate buffer solutions	Intracameral	250 $\mu\text{g.}$
Phosphate buffer solutions	Intravitreal	500 $\mu\text{g.}$
Ointments (water miscible base)	Topical	1–5

\* Given three drops every five minutes for one-half hour.

† Total amount in 0.5 ml. by subconjunctival injection.

TABLE II  
*Penetration of Spiramycin into the Aqueous of Rabbit Eyes\* with Mechanically Abraded Corneas*

Concentration of solution or ointment	Concentration of spiramycin in the aqueous, $\mu\text{g.}/\text{ml.}$ , time interval between administration of drug and aqueous withdrawal		
	1 hr.	2 hr.	4 hr.
10,000 $\mu\text{g.}/\text{ml.}$	32	16	0
20,000 $\mu\text{g.}/\text{ml.}$	48	24	12
40,000 $\mu\text{g.}/\text{ml.}$	64	48	8-12
5 per cent ointment	16-24	12	16

\* Each determination represents an average of four eyes.

tions containing 10,000, 20,000, and 40,000  $\mu\text{g.}$  of spiramycin per ml. were used. The corneas were denuded of their epithelium by mechanical abrasion of a 5 to 6 mm. area centrally using the bevel of an 18 gauge needle. The results are shown in table II. A definite penetration of spiramycin into the aqueous was detected; it will be noted that the best level in every instance was obtained after one hour and that this level had fallen by the second and fourth hour. Likewise the greater the concentration used, the greater the penetration observed during the first hour. The ointment at 5 per cent gave less penetration than any of the water solutions (1, 2, and 4 per cent), but with this mode of treatment the data suggest a tendency to maintain a constant level of antibiotic during a four hour period.

*Subconjunctival Injections of Spiramycin in Normal Eyes.* Aqueous penetration from subconjunctival injections of spiramycin solutions are represented in table III. Concentrations of 40,000, 20,000, and 10,000  $\mu\text{g.}/\text{ml.}$  were used. In each case 0.5 ml. of the test fluid was injected beneath the conjunctiva and aqueous humor was removed 1, 2, and 4 hours after injection.

A concentration of at least 20,000  $\mu\text{g.}/\text{ml.}$  was needed to effect an aqueous level of 12  $\mu\text{g.}/\text{ml.}$  This level, it will be remembered, is the minimal detectable by the method employed.

In normal eyes subconjunctival injection in the doses used produced a uniform level of antibiotic in the aqueous over a 4 hour period.

*Penetration Into Normal Eyes Following Intravenous Administration.* Single injections of solution of spiramycin in phosphate buffer were made into the ear vein of rabbits. The amount of drug injected varied between 20,000 and 200,000  $\mu\text{g.}$  Aqueous, vitreous, and/or serum were removed at intervals listed in table IV. In one instance the secondary aqueous was removed for assay. The data show that large

TABLE III  
*Penetration of Spiramycin into the Aqueous of Rabbit Eyes\* Following Subconjunctival Injection*

Concentration of solution, $\mu\text{g.}/\text{ml.}$	Concentration of spiramycin in the aqueous, $\mu\text{g.}/\text{ml.}$ , time interval between administration of drug and aqueous withdrawal		
	1 hr.	2 hr.	4 hr.
40,000	16	24	16
20,000	12	12	12
10,000	0	0	0

\* Each determination represents an average of four eyes.

TABLE IV

*Penetration of Spiramycin into Normal Rabbit Eyes\* Following Intravenous Administration*

Amount of spiramycin injected, $\mu\text{g.}$	Time interval between administration of drug and withdrawal of fluid, hour	Concentration spiramycin found, $\mu\text{g./ml.}$		
		Aqueous	Vitreous	Serum
20,000	$\frac{1}{2}$	0	0	12
100,000	$\frac{1}{2}$	24 (secondary aqueous)		
	1	0		24
	4	0		
200,000	$\frac{1}{2}$	12	0	24

\* Each determination represents an average of four eyes.

doses (in the order of 0.1 Gm./Kg. body weight intravenously) are required for aqueous penetration by intravenous route. The concentration present in the aqueous (12  $\mu\text{g./ml.}$ ) again represents the minimal detectable amount. A concentration in the blood twice this amount seems to be necessary to effect minimal aqueous levels. The vitreous fluid in these instances contained no detectable antibiotic.

*Intravenous Administration to Rabbits with Induced Ocular Inflammation.* Study was made of the ocular penetration of spiramycin under abnormal circumstances. In the first group the anterior segment of the eye was inflamed by application of 12 *N* hydrochloric acid to the cornea. A cotton applicator was dipped in the acid and held against the cornea for approximately 20 seconds following which the eye was copiously irrigated.

The second group consisted of animal eyes with induced inflammation of the posterior segment. This was accomplished by ball point surface application of diathermy to an area of sclera 4 by 10 mm. at the equator.

Intravenous injection of 200,000  $\mu\text{g.}$  spiramycin was given in divided dosage 90 minutes apart in each group. The initial dose was administered just prior to the diathermic application. Aqueous and vitreous were removed 15 minutes after the second injection. Serum samples were taken 30 minutes after the second dose.

Whether the anterior or the posterior segment of the eye is inflamed, the results indicate that, with intravenous administration (0.1 Gm./Kg. body weight), spiramycin penetrates into the aqueous but not the vitreous (see table V).

TABLE V

*Penetration of Spiramycin into Inflamed Rabbit Eyes\* Following Intravenous Administration*

Amount of drug injected	Time interval between administration of second dose and fluid withdrawal, min.	Concentration of spiramycin detected, $\mu\text{g./ml.}$				
		Anterior segment inflamed		Posterior segment inflamed		Serum
		Aqueous	Vitreous	Aqueous	Vitreous	
200,000 $\mu\text{g.}$ (given in divided dosage 90 minutes apart)	15	12	0	12	0	24
	30					

\* Each determination represents an average of four eyes.

The high sensitivity of *B. subtilis* to spiramycin suggested that this new antibiotic might be of value for the treatment of vitreous infection due to this organism. Because the vitreous penetration of spiramycin was not detectable even though large doses were given intravenously, it was decided that direct injection of the antibiotic into the vitreous might prove valuable. Infections were produced in the posterior vitreous of 52 eyes by injecting 30,000 viable organisms of *B. subtilis*. Treatment consisted of 500  $\mu$ g. of spiramycin placed into the vitreous humor of 30 rabbit eyes. The time interval between treatment and onset of infection was varied from 6 to 72 hours. Improvement of ocular inflammation was apparent only within the first 24 hours following therapy. Differences between treated and control eyes were less apparent thereafter. Objective differences based on laboratory smears and cultures were also inconclusive. It seems that the particular strain of *B. subtilis* used was unable to produce a sustained or progressive inflammation. Further evaluation might prove fruitful if a more pathogenic strain of *B. subtilis* was employed.

#### COMMENT

Based on the data presented in this paper, it can be concluded that spiramycin is well tolerated by the animal eye when administered locally or by the systemic route. Although topical application of 1 to 5 per cent solutions of this drug does not penetrate the normal eye, aqueous penetration does occur with these concentrations when the corneal epithelial barrier of the eye is damaged. Therefore, spiramycin solution in strengths ranging from 1 to 5 per cent would be expected to permeate the aqueous under those ocular conditions characterized by corneal ulceration, abrasion, or de-epithelialization whether caused by infectious, chemical, or other noxious agents. With reference to the application of ointment preparations under conditions similar to those previously mentioned, one may expect less penetration in the aqueous by a 5 per cent concentration of spiramycin in aquaphor base. Application at intervals of 2 to 3 hours for solutions and up to at least four hours for ointment form appears to be the frequency necessary to maintain detectable levels in the aqueous.

When the subconjunctival route of administration is used, observation of the data indicates that a maximal nontoxic dose of 5000  $\mu$ g. given by this method is not penetrable in the normal eye. Subconjunctival administration in eyes with abnormalities of the anterior segment was not evaluated for penetration.

Systemic use of spiramycin by intravenous injection requires rather large doses of drug in the order of 0.1 Gm./Kg. of body weight for penetration into the aqueous humor of rabbits with normal eyes. This is true even for those conditions wherein the anterior or posterior segment of the eye is inflamed. The combination of large intravenous doses and only minimal detectable penetration in the anterior segment limits the ocular effectiveness of the systemic route of administration and confines the ocular use of this drug to local application. This apparent resistance to spiramycin penetration through the normal blood-ocular barriers is somewhat analogous to the poor rate of diffusion of this antibiotic across fibrin membranes as found by the in vitro studies of Watson.<sup>11</sup> One therefore would not choose the intravenous route for therapy of ocular inflammations, which required penetration of the antibiotic into the ocular fluids. Vitreous penetration could not be achieved except by direct injection of the drug into the vitreous humor. In known human vitreal infections, due to *B. subtilis*, incomplete animal experiments in this laboratory suggest

that intravitreal injections in amounts up to 500  $\mu\text{g.}$  may be of value as a last resort in therapy. Evidence was obtained that the use of spiramycin intravitreally was effective early in the development of the infection but this observation later became less apparent due to low pathogenicity of the strain of organism.

The danger of developing resistant organisms to an antibiotic by improper and inadequate local use should be considered especially when a new antibiotic of this type becomes available for use.

#### SUMMARY

1. Spiramycin is well tolerated by the animal eye both locally and systemically.
2. Local therapy with this drug should be of value in the treatment of infections of the anterior segment of the eye caused by those gram-positive organisms sensitive to spiramycin. In addition, staphylococcal infections resistant to penicillin and other antibiotics may be amenable to this drug.
3. Both solutions and ointments are more penetrable when the corneal epithelial barrier is damaged.
4. Hypothetical dosage for topical use based on these experiments would be as follows: three drops every two to three hours for 1 to 5 per cent solutions topically; subconjunctival doses not to exceed 5000  $\mu\text{g.}$ ; ointment concentrations of 1 to 5 per cent applied every four hours.
5. Systemic administration is not recommended as a mode of therapy for ocular inflammations requiring antibiotic penetration into the ocular fluids.
6. Intravitreal injections not to exceed 500  $\mu\text{g.}$  may be of value in the treatment of *B. subtilis* vitreous infection when other methods of therapy have failed.
7. Inadequate and improper local ocular therapy may reduce the value of this antibiotic for other systemic infections by developing organisms resistant to this drug.

#### BIBLIOGRAPHY

1. PINNERT-SINDICO, S.; NINET, L.; PREUD'HOMME, J., AND COSAR, C.: A new antibiotic—spiramycin. In: Antibiotics Annual 1954–1955. New York, Medical Encyclopedia, Inc., 1955; pp. 724–727.
2. LAUBIE, M. H.: Identification de la spiramycine, Bull. Soc. Pharm. Bordeaux 95:12, 1956.
3. CHABBERT, Y.: Études in vitro sur la spiramycine; activité, résistance, antibiogramme, concentrations humérales. Ann. Inst. Pasteur 89:434–446, 1955.
4. DI MARCO, A., AND GHIONE, M.: Ricerca sull'attività antibatterica di un nuovo antibiotico: La spiramicina (Rovamicina), Atti Sc. Lomb. Sc. Med. 10:102–106, 1955.
5. CORBAZ, E. L.; GAUMANN, E.; KELLER-SCHIERLEIN, W.; KRADOLFER, F.; KYBURZ, E.; NEIPP, L.; PRELOG, V.; WETTSTEIN, A., AND ZAHNER, H.: Stoffwechselprodukte von Aktinomyceten. 4. Mitteilung. Die Foromacidine A, B, C, and D, Helvet. chim. acta 39:304, 1956.
6. FINLAND, M.: Current concepts in therapy: Antibiotics. I. General consideration and range of activity, New England J. Med. 258:85–86, Jan. 9, 1958.
7. JONES, W. F., JR., AND FINLAND, M.: Antibiotic combinations. Tetracycline, erythromycin, oleandomycin and spiramycin, and combinations of tetracycline with each of the other three agents—comparisons of activity in vitro and antibacterial action of blood after oral administration, New England J. Med. 257:481–491, Sept. 12, 1957.
8. JONES, W. F., JR., AND FINLAND, M.: Antibiotic combinations (concluded). Tetracycline, erythromycin, oleandomycin and spiramycin and combinations of tetracycline with each of the other three agents—comparisons of activity in vitro and antibacterial action of blood after oral administration, New England J. Med. 257:536–547, Sept. 19, 1957.
9. RENNOUX, G.; DUPOUX, R., AND HUET, M.: Premier résultats d'essais de la "spiramycine," Arch. Inst. Pasteur de Tunis 32:337–343, 1955.
10. BOYD, E. M.: The acute oral toxicity of spiramycin, Canad. J. Biochem. & Physiol. 36:103–110, Jan., 1958.
11. WATSON, K. C.: The "In vitro" diffusion of antibiotics through fibrin membranes, Brit. J. Exper. Path. 38:493–497, Oct. 1957.

# Apparent Paradox of Antimicrobial Activity of Spiramycin

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In deciding which of several possible antibiotics to use, the clinician must consider their *in vitro* activity as determined by antibiogram and the serum levels they can produce. However, experimentation has shown that the *in vivo* activity of an antibiotic cannot be exactly predicted from its *in vitro* activity and from blood levels.

Spiramycin in particular, although less active *in vitro* than other antibiotics of the same group, has an *in vivo* activity on experimental infections of the mouse equal or superior to that of the other antibiotics. Moreover, it is effective with infections that require, for inhibition, *in vitro* concentrations of spiramycin equal or superior to the attainable blood levels. Videau and Jonchere<sup>8,9</sup> had shown that spiramycin attached itself to the bacteria, but this observation did not seem to explain fully the apparent contradiction.

The present work suggests an explanation for this paradox and at the same time draws attention to an undoubtedly important factor of antibiotic activity: its concentration in tissues. It describes the results of antimicrobial activity tests in mice and titration of antibiotic levels in various organs.

*In Vivo Activity Tests.* We used chemotherapeutic tests differing from those habitually employed by the mode of infection, the conditions of treatment, and the use of a strain made spiramycin resistant.

**MODE OF INFECTION.** We tried to bring about, by intravenous or intramuscular injection of bacteria to mice without addition of mucin, infections that were less painful, more like spontaneous illnesses than those of intraperitoneal infection, less rapidly generalized than the latter, and that caused elective visceral localizations. We chose renal staphylococcic and intramuscular streptococcic infections of the mouse. We thought that these tests, if they gave results differing from those obtained by classical methods, would bring to light differences in the mechanism of *in vivo* activity of antibiotics.

**CONDITIONS OF TREATMENT.** We infected the animal several hours after the administration of antibiotics, at a time when the antibiotic blood level was low, thinking that it would thus be possible to evaluate the eventual persistence of active antibiotic in the organism.

**USE OF A STRAIN MADE RESISTANT TO SPIRAMYCIN.** By the use of such a strain we hoped to determine whether a strain necessitating large *in vitro* concentrations for its inhibition was nevertheless sensitive *in vivo*.

*Antibiotic Titration in Organs of the Mouse.* Titrations of antibiotics in different organs of the mouse were carried out at various times after oral administration of these antibiotics; the blood levels were determined simultaneously.

## IN VIVO ANTIMICROBIAL ACTIVITY TESTS

*Material and Methods.* **RENAL STAPHYLOCOCCIC INFECTION OF THE MOUSE.** *Staphylococcus* strain 133 of the Pasteur Institute was used. Infection was by intravenous injection of 0.15 ml. of an 18 hour culture in peptone-glucose-phosphate medium. With the product in gum suspension, an oral dose was administered in 1 ml./20 Gm.; three to four treatments were given at 24 hour intervals, the first immediately after the infection; there were 10 mice/dose.

The surviving mice were killed on the tenth day, and we performed autopsy on all mice (those that died during the test and those killed at the end) and examined the macroscopic renal lesions.

The untreated control mice died after two to three days. They always showed small renal abscesses when death occurred more than 24 hours after inoculation; these lesions were not always apparent when death occurred prematurely. At the end of the test, it was possible to observe large abscesses in treated mice; to calculate the  $CD_{50}$ , we considered only the survival of the animals, not their renal lesions. These are only a means of estimating roughly the activity of the studied product; however, it is possible to determine the dosage of antibiotic that stops their appearance.

We must emphasize, as did Chabbert et al,<sup>1</sup> the importance of the choice of strain; pathogenic staphylococci, which are perfect for an intraperitoneal infection of the mouse, cannot be used for an intravenous infection. Although all staphylococci have a special affinity for the kidneys, there are considerable differences from one strain to another in time of appearance of the abscesses, their size, and the time of survival of the animals. Smith et al<sup>4</sup> have shown that an intravenous inoculation of 0.1 ml. of an undiluted culture of Smith staphylococci kills only 30 per cent of the mice in one month; on the contrary, the Giorgio strain at the same dosage kills all the animals in approximately one week. Our Smith strain, whose pathogenic power is comparable to that of the 133 strain by the intraperitoneal route, does not cause the death of all the mice after 15 days, even when injected at the large dosage of 0.5 ml. It cannot therefore be used for quick chemotherapeutic tests, whereas the 133 *Staphylococcus* is ideal for this purpose.

**INTRAMUSCULAR STREPTOCOCCIC INFECTION OF THE MOUSE.** *Streptococcus* strain Dig. 7 was used. Infection was by intramuscular injection of 0.2 ml. of an 18 hour culture in a brain-heart infusion medium diluted to  $10^{-4}$  in saline solution. With the product in gum suspension, an oral dose was administered in 1 ml./20 Gm.; three treatments were given at 24 hour intervals, the first at 24 hours after infection; there were 10 mice/dose. Observation was for eight days.

The untreated control mice had a serious edema of the thigh and died within four days after infection.

**PREVENTIVE TREATMENT OF THE RENAL STAPHYLOCOCCIC INFECTION OF THE MOUSE.** A single oral administration of the antibiotic was given. Infection was four to six hours after an intravenous injection of 0.15 ml. of a culture of *Staphylococcus* 133. The average lifespan of the animals was calculated and compared to that of untreated controls infected at the same time.

**TREATMENT OF A STAPHYLOCOCCIC INFECTION DUE TO STRAINS MADE SPIRAMYCIN-RESISTANT.** Infection and treatment were as with the renal staphylococcic infection, but the resistance of the *Staphylococcus* 133 used was increased by the method of successive transfers. It was sensitive only to 50  $\mu$ g./ml. of spiramycin.

**Results.** We compared the activity of spiramycin, erythromycin, carbomycin, oleandomycin, and triacetyloleandomycin on the classical intraperitoneal infection of the mouse by staphylococci or streptococci\* and on the intravenous staphylococ-

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\* Intraperitoneal infection by 133 *Staphylococcus*: 0.5 ml. of a  $2 \times 10^{-3}$  dilution in 5 per cent mucin of an 18 hour culture of staphylococci in peptone-glucose-phosphate medium; oral treatment started immediately after infection; one, three, or four treatments at 24 hour intervals. Observation for eight days.

Intraperitoneal infection by *Streptococcus* Dig. 7: 0.5 ml. of a  $10^{-3}$  dilution in saline solution of an 18 hour culture in serum broth; treatment carried out as for staphylococcic infection.

TABLE I

*Comparison of the Activity of Oral Spiramycin, Erythromycin, and Carbomycin on Intraperitoneal and Intravenous Staphylococcic and Intraperitoneal and Intramuscular Streptococcic Infections of the Mouse*

Organism	Route of infection	Number of treatments	CD <sub>50</sub> in mg./Kg./day orally of:		
			Spiramycin	Erythromycin	Carbomycin
<i>Staphylococcus</i> 133	Intraperitoneal	4	125	90	220
		3	115		200
	Intravenous	4	250	485	>1000
		4	310	>500	
		3	250	460	
<i>Streptococcus</i> Dig. 7	Intraperitoneal	3	85	135	415
	Intramuscular	3	130	>500	>1000
		3	345	>1000	

cic and intramuscular streptococcic infections, the microbial strains used for the two types of infection being of course the same: 133 *Staphylococcus* and Dig. 7 *Streptococcus*.

Tables I and II show the CD<sub>50</sub> of the different antibiotics on the four types of infection. Spiramycin is seen to be more active than carbomycin but less active in these tests than erythromycin, oleandomycin, or triacetyloleandomycin on the intraperitoneal staphylococcic infection; it was most active on an intravenous staphylococcic infection. The good activity of spiramycin is thus even more obvious in the case of intravenous infection than intraperitoneal infection. It is important also to note the absence of renal staphylococcic lesions in most of the surviving mice treated with spiramycin, whereas the animals treated with other antibiotics nearly all showed the presence of renal abscesses. Spiramycin, more active than the other antibiotics in the intraperitoneal streptococcic infection, was even more effective in the treatment of intramuscular streptococcic infections.

It appears therefore that the relative activity of antibiotics *in vivo* varies with the mode of infection, which conditions the localization of the infectious process. This leads to the conclusion that the concentration of antibiotic at the site of attack of the germ is doubtlessly an important factor in its activity and that spiramycin probably attains effective levels in the tissues, particularly in the kidneys.

Tables III and IV show the comparative preventive activities of spiramycin, erythromycin, carbomycin, oleandomycin, and triacetyloleandomycin on the intra-

TABLE II

*Comparison of the Activity of Oral Spiramycin, Oleandomycin, and Triacetyloleandomycin on Intraperitoneal and Intravenous Staphylococcic and Intraperitoneal and Intramuscular Streptococcic Infections of the Mouse*

Organism	Route of infection	Number of treatments	CD <sub>50</sub> in mg./Kg./day orally of:		
			Spiramycin	Oleandomycin	Triacetyl-oleandomycin
<i>Staphylococcus</i> 133	Intraperitoneal	3	198		45
		3	115	20	
	Intravenous	4	375		>500
		3	415	>500	
<i>Streptococcus</i> Dig. 7	Intraperitoneal	3	130	355	
		3	87		470
	Intramuscular	3	345	>500	>500
		3	175	>1000	

TABLE III

Preventive Effect of Spiramycin, Erythromycin, and Carbomycin on Intravenous Staphylococcal Infections of the Mouse

Spiramycin, 500 mg./Kg. orally										Erythromycin, 500 mg./Kg. orally								Carbomycin, 500 mg./Kg. orally																					
Infection after 4 hr., day										Infection after 4 hr., day								Infection after 4 hr., day																					
1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
V	V	+	*							V	+									V	+									V	+								
V	V	+								V	+									V	+									V	+								
V	V	+	+							V	+									V	+									V	+								
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V	V	+	+							V	+									V																			

TABLE IV

*Preventive Effect of Spiramycin, Oleandomycin, and Triacetyloleandomycin on Intravenous Staphylococcal Infections of the Mouse*

Spiramycin, 500 mg./Kg. orally										Oleandomycin, 500 mg./Kg. orally								Triacetyloleandomycin, 500 mg./Kg. orally																				
Infection after 4 hr., day										Infection after 6 hr., day								Infection after 4 hr., day								Infection after 6 hr., day												
1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	1	2	3	4	5	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
V	V	V	V	+	*					V	V	+						V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
V	V	V	V	V	V	V	V	V	V	V	V	V	+					V	V	+																		
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venous staphylococcic infection of the mouse. The activity tests were carried out four to six hours after oral administration of the antibiotic. They show that spiramycin increases the survival time of mice infected four to six hours after its administration; under the same conditions, the other antibiotics have a very weak action, carbomycin having no effect at all. This fact can apparently be explained only by the presence of active spiramycin in the organism at least six hours after its administration. Since blood titrations show that at that time the serum concentration scarcely reaches the minimum inhibitory concentration (MIC) for the 133 *Staphylococcus*, it is logical to assume that spiramycin persists in the tissues.

During our tests intravenous infection of the mouse with a strain of *Staphylococcus* 133 sensitive to 50  $\mu$ g./ml. of spiramycin, we found a  $CD_{50}$  of 850 mg./Kg./day orally of spiramycin, whereas it was 310 mg./Kg./day orally for the infection obtained with the initial strain. Thus an antibiotic, spiramycin in particular, can be active in vivo on germs necessitating for their inhibition in vitro concentrations greatly superior to those that can be obtained in the blood.

Titrations carried out in the viscera of mice confirmed the presence and persistence at high levels of spiramycin in the organism.

#### TITRATION OF ANTIBIOTICS IN THE VISCERA

*Material and Methods.* One hundred, 250, or 500 mg./Kg. of antibiotics was administered in a single oral dose to a group of 10 mice as 1 ml./20 Gm. of gum suspension; 6, 24, or 48 hours later, the animals were killed. The spleen, the kidneys, the lungs, the heart, and the liver were removed. The organs were grouped and carefully separated from the accompanying blood; each group was weighed so that the results obtained could be expressed relatively to 1 Gm. of viscera. Each result is therefore the mean of 10 results, since it represents the organs of 10 sacrificed mice.

**EXTRACTION OF THE ANTIBIOTIC.** The five antibiotics studied here can be extracted by analogous methods. For an alkaline pH (approximately 9), they are free bases and are then very soluble in methanol. The extraction solution we used was a mixture of 70 per cent methanol and 30 per cent universal Britton buffer, pH 9.2.

The extraction technique was the same for each set of organs. These were carefully crushed in a mortar with Fontainebleau sand (1 Gm./2 Gm. of viscera). Twenty ml. of solvent were added in fractions, while crushing was continued. The complete mixture of viscera, sand, and solvent was then placed in a stoppered Erlenmeyer flask. The flask was placed on a shaker for one hour, and the mixture was then rapidly filtered on a Büchner under vacuum; a small quantity of solvent was used to rinse the flask and the filter, and the volume was adjusted to 25 ml.

The quantity of solvent used was the same (25 ml.) for the spleen, the kidney, the lung, and the heart. For the liver, which is much larger, we were forced to use a considerably greater quantity of solvent. The filtrate obtained was kept in a stoppered flask and was diluted, if necessary, with the solvent mixture before titration.

**TITRATION.** We used the method of titration by diffusion. Because of its great sensitivity to these antibiotics, *Sarcina lutea* was chosen as test organism, thus permitting the detection of relatively small levels.

Filter paper discs of 12 mm. diameter were soaked in the solutions to be analyzed and then left to dry for two hours on glass slides. Other discs were soaked in corresponding standard solutions, in the same solvent, and left to dry in a similar manner. All the discs thus prepared were placed on gelose medium dishes inoculated with *S. lutea*, and titration was effected by the classical method.

TABLE V

*Concentrations in  $\mu\text{g./Gm.}$  of Spiramycin, Erythromycin, Carbomycin, Oleandomycin, and Triacetyloleandomycin in the Organs of Mice and in  $\mu\text{g./ml.}$  in the Blood; single oral administration of 100, 250, or 500 mg./Kg. of antibiotic*

Organ	Dose, mg./Kg. orally	Spiramycin, levels after, hours*			Erythromycin, levels after, hours		Carbomycin, levels after, hours		Oleando- mycin, levels after, hours		Triacetyl- oleandomycin, levels after, hours	
		6	24	48	6	24	6	24	6	24	6	24
Spleen	100	22	<5		0.8		<2		11		16	<4
	250	71	25	10	7		<2		17		56	<4
	500	122	164	26	80	6	97	<2	114	4	108	<4
Kidney	100	10	<2		<0.5		<1		7		22	<2
	250	37	12	5	5		<1		11		44	<2
	500	107	108	15	51	<0.5	7	<1	52	<1	80	<2
Lung	500	87	103	8	43	<0.5	18	<1	42	<1	113	2
Heart	500	56	46	5	2.5	2	6	<3	22	3	40	<4
Liver	500	150	32	12	37	<0.5	3	<1	170	<1	165	2
		2*	6		2	6	2	6	2	6	2	6
	250	6.7			7.4		8.7		2.2		5	
Blood	500	11.2	3.9		16	5	16	7.2	8.6	1.8	10.8	7.3

\* Time between the time of administration of antibiotic and the time of removal of the organ.

**Results.** The results are given in table V and figure 1, which shows clearly the great differences that exist between the five antibiotics compared.

Six hours after oral administration, spiramycin had the highest concentrations in most of the organs; its level was 20 times greater than that of erythromycin in the myocardium, 50 times that of carbomycin in the liver. Oleandomycin and triacetyloleandomycin have levels of the same order as spiramycin in the spleen and in the liver; triacetyloleandomycin has a higher level in the lungs. In the kidneys, which is the area of particular interest here since its antibiotic level could explain the activity on intravenous staphylococcal infections, the concentration of spiramycin is twice that of erythromycin or oleandomycin and seven times that of carbomycin.

The differences become very important 24 hours after administration; except in the case of spiramycin, which maintains its six hour levels in all viscera except the liver, it is almost impossible to detect the other antibiotics in the organs. Forty-eight hours after a single oral dose, spiramycin is still detectable in the organs and the levels reached are higher than the highest attainable levels in the blood. Carbomycin is remarkable for its low and short-lasting tissular concentrations.

These results confirm and complete those obtained by Chabbert et al.,<sup>1</sup> who found in mouse kidneys a concentration of spiramycin four times greater than that of erythromycin, those obtained by Pellerat and Maillard,<sup>3</sup> who showed the persistence of spiramycin in guinea pig organs after prolonged treatment; those of Kazenko et al.,<sup>2</sup> who emphasized the high oleandomycin levels in the organs of monkeys and rats three to four hours after oral administration of that antibiotic. Spitzzy and Hitzenberger,<sup>7</sup> by early titration of various antibiotics in the blood after intravenous injection, deduced that some were retained by the tissues in much smaller quantities than others. These results also show that the activity of spiramycin in the organism is quite different from that of the other antibiotics, since it alone has the double advantage of high and long-lasting tissular concentrations.

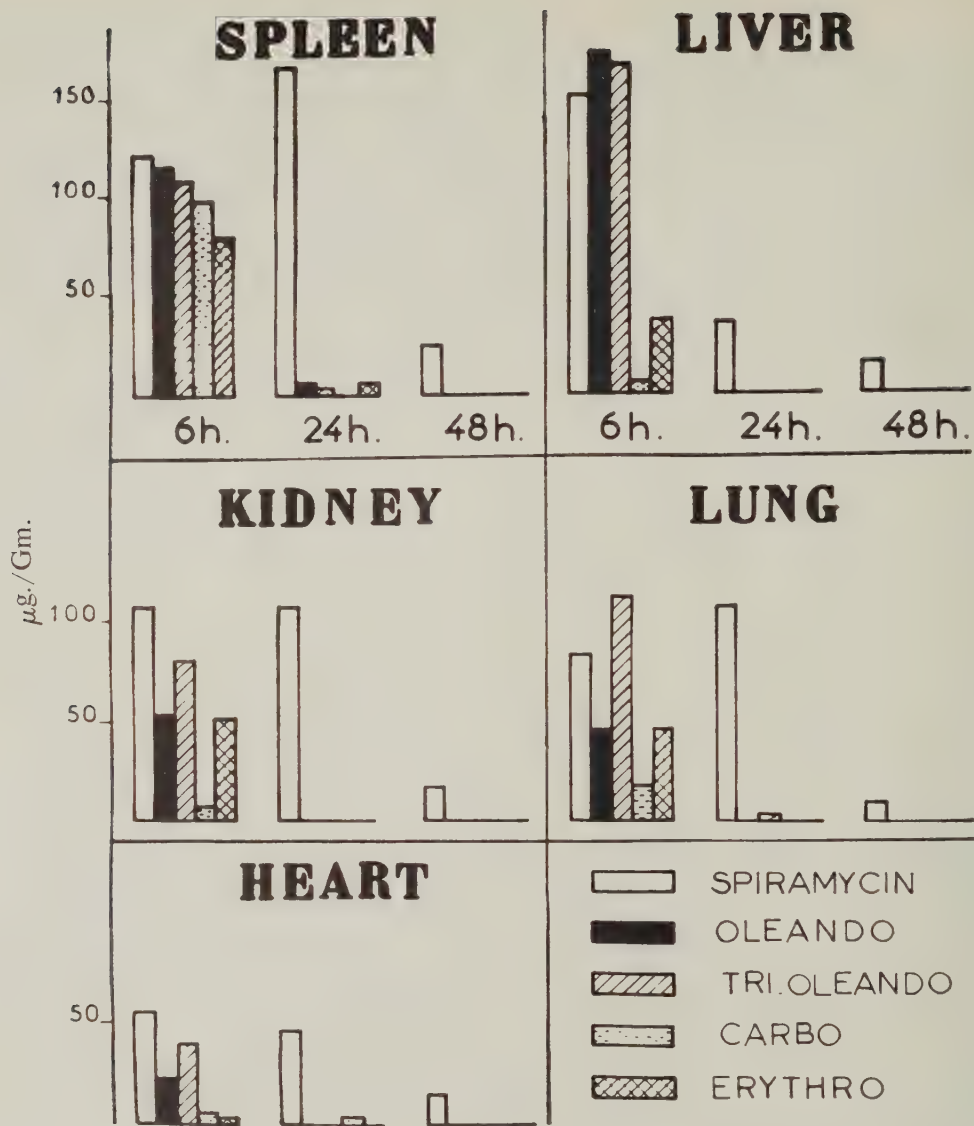


FIG. 1. Concentration of the various drugs are shown in the organs of mice given a single dose orally of 500 mg./Kg.

#### DISCUSSION AND CONCLUSIONS

Spiramycin, which is less active in vitro on staphylococci and streptococci than the other antibiotics of the same group, is more active than the others on intravenous staphylococcal infection in the mouse and on intraperitoneal and intramuscular streptococcal infections. Its blood levels are of the same order as those of the other antibiotics. But it differs from the others by its distribution in the organism, its high tissular concentration, its persistence in the viscera.

We suggest that this special activity of spiramycin is a possible explanation of the discrepancy between its in vivo antimicrobial action and its in vitro activity. It is capable of prolonging the life of animals infected with staphylococci whose sensitivity is at the limit of attainable spiramycin in the blood; it has a preventive effect, although sometimes only partial, at a time when blood concentration is low; it is superior to the other antibiotics for an infection essentially localized in an organ in which high concentrations are reached.

But more generally, this study draws attention to the probable part played by the tissular concentrations of antibiotics in their activity. It is obvious, then, that an examination of the in vitro bacteriostatic activity of an antibiotic and its concentration in the blood is not sufficient to predict its in vivo activity. Thus the  $CD_{50}$  of spiramycin on renal staphylococcal infection of the mouse is about 250 mg./Kg. orally, a dosage that produces blood levels after two hours of 6.7  $\mu\text{g./ml.}$ , i.e., a concentration equal to approximately twice the MIC of spiramycin for the staphylococci responsible for the infection. Fifty per cent of the treated animals survived with this dosage, whereas the administration of 250 mg./Kg. of erythromycin, which produces a blood concentration of 7.4  $\mu\text{g./ml.}$ , or approximately 35 times the MIC of erythromycin, was not effective.

Other factors must then be considered to explain the activity of an antibiotic; among these, tissular concentrations and their variation with time are undoubtedly important. De Somer et al<sup>5,6</sup> noted the parallelism between the activity of the erythromycin and carbomycin group of antibiotics and their concentrations in the tissues.

In practice, then, we should consider the antibiogram useful in guiding treatment, but since it represents only one activity factor, it must not be used as an absolute criterion. The tissular affinity of antibiotics should also be taken into consideration, together with the blood levels they can produce.

In this respect, spiramycin is remarkable for ability to attain high and lasting tissular concentrations in the animal.

#### SUMMARY

Spiramycin was found to be more active in vivo than would be expected from in vitro activity tests and study of blood level concentrations. The present work suggests an explanation to this paradox. We have shown that the efficacy of spiramycin in renal staphylococcal infections in the mouse, i.e., an experimental disease less severe and more localized than the classical peritoneal infection, is greater than that of other antibiotics (carbomycin, erythromycin, oleandomycin, triacetyloleandomycin), which are more active in vitro on *Staphylococcus*.

It was also observed that a single oral administration of spiramycin in mice affords definite protection against an intravenous injection of *Staphylococcus* given after a few hours, at a time when the antibiotic blood level is low. These observations seem to prove, and this is confirmed by titration, that spiramycin attains high and persistent concentrations in the tissues. These concentrations might condition the in vivo activity.

Attention is drawn to the insufficiency of in vitro activity tests and measurement of blood levels in judging the value of an antibiotic; the levels that occur in the tissues should also be considered. From this point of view, spiramycin is remarkable in its ability to attain high and lasting tissular concentrations. These findings may have a practical application in clinical medicine.

#### BIBLIOGRAPHY

1. CHABBERT, Y.; BOYER, F.; SAVIARD, M.; BOULINGRE, H., AND HERVE, J.: Détermination de l'action bactéricide in vivo des antibiotiques dans la staphylococcie rénale de la souris, Ann. Inst. Pasteur 92:760-777, 1957.
2. KAZENKO, A.; SORENSON, O. J., JR.; WOLF, L. M.; DILL, W. A.; GALBRAITH, M., AND GLAZKO, A. J.: Physiologic disposition of oleandomycin in animals, Antib. & Chemo. 7:410-418, 1957.

3. PELLERAT, J., AND MAILLARD, M. A.: Fixation tissulaire de la spiramycine chez le cobaye. Comparaison avec d'autres antibiotiques. *Compt. rend. Acad. Sc.* 247:894-896, 1958.
4. SMITH, J. M., AND DUBOS, R. J.: The behavior of virulent and avirulent staphylococci in the tissues of normal mice, *J. Exper. Med.* 103:87-108, 1956.
5. DE SOMER, P.; VAN DIJCK, P., AND VAN DE VOORDE, H.: Griseomycin tissue concentrations following parenteral and oral administration, *Antib. & Chemo.* 4:546-550, 1954.
6. DE SOMER, P., AND VAN DIJCK, P.: A preliminary report on antibiotic number 899, a streptogramin-like substance, *Antib. & Chemo.* 5:632-639, 1955.
7. SPITZY, K. H., AND HITZENBERGER, G.: The distribution volume of some antibiotics. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 996-1003.
8. VIDEAU, D., AND JONCHERE, H.: Sur une modalité particulière d'action de la spiramycine *in vitro*, *Ann. Inst. Pasteur* 92:202-211, 1957.
9. VIDEAU, D.: Sur le mode d'action des antibiotiques. Cas particulier de la spiramycine, *Ann. Inst. Pasteur* 94:709-723, 1958.

# The Effect of Antibiotics of the Tetracycline Group on Enzymes and the Practical Clinical Significance Thereof

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This report is based on the experimental observation that chlortetracycline inhibits pancreatic enzymes. The following basic observations were made: (1) Tetracyclines, and especially chlortetracycline, inhibit the activity of pancreatic  $\alpha$ -amylase and lipase. (2) This inhibitory effect is due to the presence of di- and multivalent metals, especially calcium and magnesium ions. Under optimal conditions, the inhibition of enzymic activity can be shown to occur in the presence of concentrations of the antibiotic as low as 10  $\mu$ g. and less per ml. (3) The inhibitory effect of chlortetracycline on these enzymes can be reversed by some organic acids, especially citric acid, and for the reversal of the inhibitory effect, the optimal weight ratio is 5 parts of citric acid per 1 part of chlortetracycline.

Some of these findings are summarized in the figures. Figure 1 shows the effect of calcium ions on the inhibition of pancreatic amylase by chlortetracycline. It can be seen that the effect of chlortetracycline is demonstrable only in the presence of calcium. The same relationship was found with lipase.

Figure 2 demonstrates the effect of citric acid on the inhibition of lipase by chlortetracycline. It shows clearly that on addition of citric acid, the activity of lipase, inhibited by chlortetracycline, is re-established. The same is true for  $\alpha$ -amylase.

The practical significance of these observations is the subject of this report. In practice the effect of chlortetracycline on enzymes manifests itself in two respects: unfavorably, where the presence of active enzymes is required for physiological processes in the organism, and favorably, where the activity of enzymes forms part of the pathogenesis of some diseases.

The unfavorable effect of chlortetracycline on enzymes manifests itself especially on the gastrointestinal tract when tetracyclines are given orally, provided their concentration is sufficient to inhibit the activity of pancreatic enzymes (lipase,  $\alpha$ -amylase). There are several consequences of this effect. In the presence of chlortetracycline the intestinal absorption of those compounds is decreased or inhibited, for the absorption of which the presence of active digestive enzymes is essential. Citric acid, given at the optimal weight ratio of 5 parts to 1 part of chlortetracycline, almost completely restores the absorption of such compounds. Some of these facts are demonstrated in the figures summarizing experimental results obtained on dogs. Figure 3 shows that in the presence of chlortetracycline the glycemic curve after feeding of glucose or starch is significantly lowered. Citric acid restores the intestinal glucose absorption. On the other hand, the absorption of para-aminosalicylic acid remains almost unaffected by chlortetracycline.

Figure 4 shows these relationships for the absorption of lipids labeled with  $C^{14}$ .

The question must be raised of whether the limited absorption of chlortetracycline itself may not be related to the inhibition of the activity of pancreatic enzymes. Such a possibility is borne out by the fact that the same compounds that increase the effect

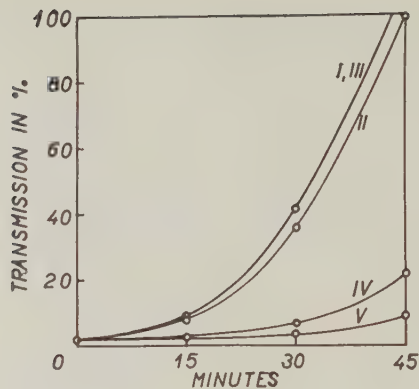


FIG. 1. The effect of calcium on the inhibition of pancreatic  $\alpha$ -amylase by chlortetracycline is shown. I, control without chlortetracycline and calcium chloride; II, 500  $\mu\text{g./ml.}$  chlortetracycline, without calcium chloride; III, calcium chloride/ $2.10^{-2}M$ , without chlortetracycline; IV, 250  $\mu\text{g./ml.}$  chlortetracycline plus  $2.10^{-2}M$  calcium chloride; V, 500  $\mu\text{g./ml.}$  chlortetracycline plus  $2.10^{-2}M$  calcium chloride. The activity is expressed in per cent transmission of the starch-iodine complex, as compared with the standard.

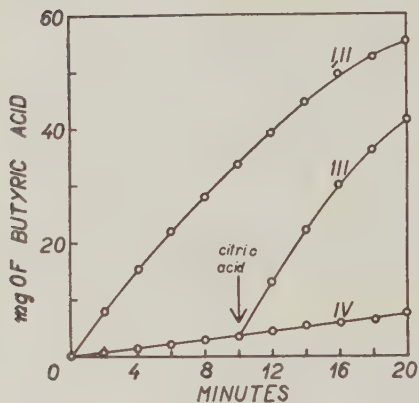


FIG. 2. The effect of citric acid on the inhibition of lipase by chlortetracycline is illustrated. I, control/ $1.10^{-3}M$  calcium chloride; II, 200  $\mu\text{g./ml.}$  chlortetracycline plus  $1.10^{-3}M$  calcium chloride plus  $10^{-2}M$  sodium citrate/ $\text{pH } 6.1$ ; III, 200  $\mu\text{g./ml.}$  chlortetracycline plus  $1.10^{-3}M$  sodium citrate/final concentration  $10^{-2}M$  added at 10 min.; IV, 200  $\mu\text{g./ml.}$  chlortetracycline plus  $1.10^{-3}M$  calcium chloride. The activity is expressed in mg. of butyric acid liberated from tributyrine.

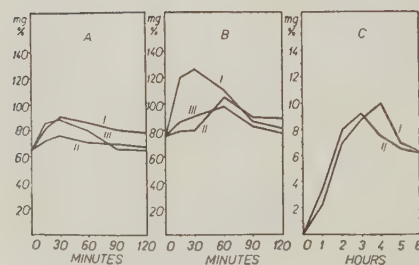


FIG. 3. The effect of chlortetracycline on the glucose level in the blood after feeding of glucose (A) 1 Gm./Kg. and starch (B) 5 Gm./Kg. and on the level of PAS in blood (C) after oral application of 0.2 Gm./Kg. in dogs is shown. I, control curves after application of the compounds without chlortetracycline; II, values after application of the compounds together with chlortetracycline; III, values after application of the compounds with chlortetracycline and citric acid.

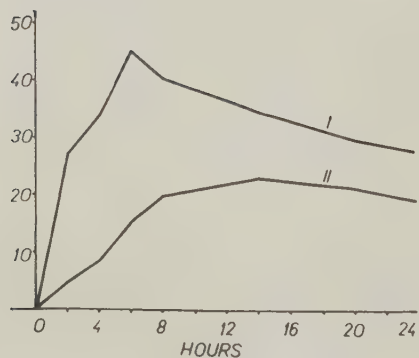
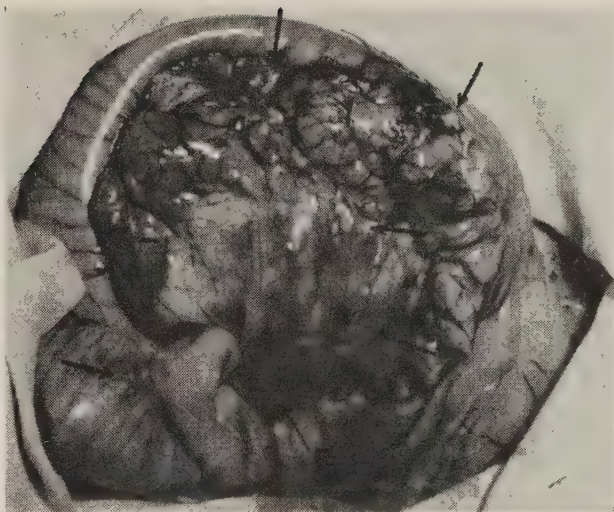


FIG. 4. The effect of chlortetracycline on the absorption of lipids ( $C^{14}$ ) in dogs is shown. I, control curves after application of lipids without chlortetracycline; II, values after application of lipids with chlortetracycline.

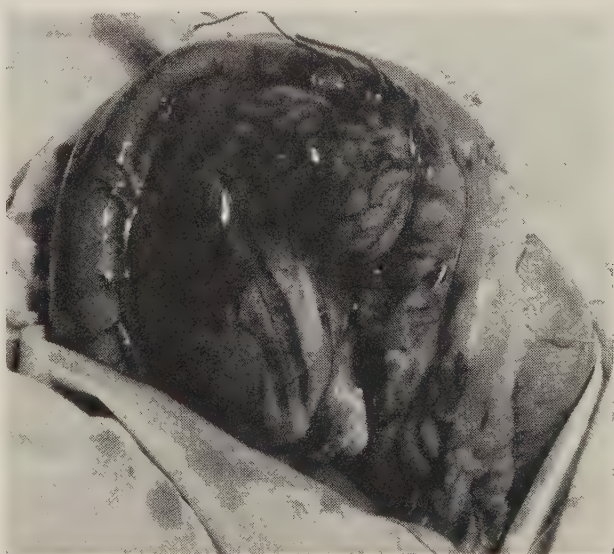
FIG. 5. The protective effect of chlortetracycline on the formation of fatty necroses during experimental acute necroses of the pancreas in dogs is illustrated. Shown is a photogram of the pancreas without application of the drug.



of chlortetracycline on enzymes, i.e., calcium or magnesium ions, also decrease the absorption of chlortetracycline, and that those compounds that abolish the effect of chlortetracycline on the enzymic activity, i.e., citric acid, increase the absorption of chlortetracycline. The following observation is also in accord with such a view: In experiments with dogs and also in clinical tests on patients, the greatest increase of chlortetracycline concentration in the blood was found when citric acid was given in that weight ratio to chlortetracycline that was found to be optimal for the reversal of chlortetracycline action on enzymes.

It is evident, however, that the effect of tetracyclines on the digestive enzymes contributes to the well-known secondary manifestations found after their oral application. All disturbances found after application of the drug, such as nausea, diarrhea, and steatorrhea, are identical to those observed by the clinician in the course of insufficient external pancreatic secretion, e.g., during an attack of chronic pancreatitis. The lowering of the glycemic curve is also common to both these syndromes. We suggest that the secondary manifestations after chlortetracycline arise not only from a decrease of microbial flora and direct irritation of the intestinal

FIG. 6. This is a photogram of the pancreas with application of chlortetracycline.



epithelium by the antibiotic, but mainly from a disturbance of physiological processes in the intestine as a consequence of the inhibition of digestive enzymes.

We therefore gave patients chlortetracycline together with citric acid and noted the occurrence of undesirable secondary manifestations. We found that the addition of citric acid reduced the occurrence of secondary manifestations to a minimum; thus, of 80 children receiving chlortetracycline for a prolonged period, only 1 had nausea.

A favorable effect of chlortetracycline on enzymes was most clearly demonstrable in experimental acute pancreatic necrosis in dogs. This disease was chosen because of the well-known role of activated pancreatic lipase in the pathogenesis of this disease, and especially its role in the formation of fatty necroses.

Acute necrosis of the pancreas was produced in 18 dogs by simultaneous ligation of the pancreatic and thoracic ducts. Changes of the pancreas and surrounding tissue were noted after 24, 48, and 72 hours macroscopically, histologically, and with roentgenograms. In order to achieve high antienzymic concentrations of chlortetracycline in the pancreatic tissue and thus to ensure as far as possible an effect of chlortetracycline on lipase activity, the antibiotic was applied directly into the pancreatic duct (10 mg. in 10 ml. of physiological saline). In control animals, 10 ml. of physiological saline was introduced into the pancreatic duct.

The results obtained in all groups of animals provide evidence for the view that chlortetracycline, given as described previously, completely protects the pancreas and surrounding adipose tissue against the formation of fatty necroses. In all control animals, in addition to tissue edema, a considerable amount of extensive fatty necrosis was found, localized not only in the pancreatic tissue but also in the adipose tissue of the omentum and mesentery, especially along the lymphatic vessels and nodes (fig. 5). On the other hand, in animals after application of the drug, necroses were almost never found. In some cases isolated small necroses were observed in the vicinity of the ligated duct (fig. 6).

The results of these experiments show clearly that the antienzymic effect of chlortetracycline may be of use in experimental acute necrosis of the pancreas.

The question should be considered whether the favorable effect of chlortetracycline on the course of posthemorrhagic shock and generally on tissues exposed to hypoxia might not be related to its antienzymic effect. The following observation suggests such a view: In experiments on hemorrhagic shock in dogs, best results were obtained in those cases where chlortetracycline was deliberately applied to the portal circulation in order to achieve high antienzymic concentrations of chlortetracycline in the liver. For these reasons the problem of the relationship of tetracyclines and enzyme activity is being studied further.

In conclusion, it may be stated that the study of the effect of tetracyclines on enzymes has shown some new, hitherto unexplored relationships of practical significance.

# The Effectiveness of Glucosamine-Potentiated Tetracycline in Upper Respiratory Infections in Children

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The past few years have seen a concentration of research efforts on a relatively new aspect of antibiotic therapy, the absorption-enhancing agent. A number of such agents, combined with tetracycline and oxytetracycline, have been evaluated, the most recent of which is glucosamine. The scope of the individual studies comparing these agents has varied, as well as the conclusions; however, recently in a four way crossover study, including 40 persons, it was reported that the oral administration of glucosamine with tetracycline (Cosa-Tetracyn\*) effected antimicrobial levels superior to those obtained from any of the enhancement formulas currently available (tetracycline phosphate complex, tetracycline plus citric acid, and tetracycline plus sodium hexametaphosphate).<sup>1</sup>

It is timely to note at this point that one of the most interesting and, perhaps, most useful parallel developments associated with studies related to the increased absorption of antibiotics has been the evolution of a new technique to measure antibiotic serum levels. Basically this procedure, developed by Snell and Garkuscha,<sup>2</sup> involves the labelling of the tetracycline or oxytetracycline molecule so that measurement of the antibiotic serum levels is determined by radiation count. A number of rather obvious advantages follow from this procedure. While errors inherently present in microbiological technique tend to "even out" in larger studies, this is not necessarily the case with a limited evaluation. Analysis by radiation count is not subject to such errors. Greater precision and freedom from such interfering factors as ". . . antagonistic effects, chelation, serum protein binding, absorption from cellular elements of the blood . . ." is now possible. By this procedure, it has been found that glucosamine-enhancement of antibiotic absorption in dogs and mice has ranged to as high as 2.4 times that of the antibiotic alone. But where does absorption enhancement lead us from a clinical standpoint? What are the facts? Where do the advantages lie?

Recently, glucosamine with tetracycline has proved to be clinically effective in gynecological infections,<sup>3</sup> upper respiratory infections in adults,<sup>4</sup> and children,<sup>5</sup> and dermatological infections or secondarily infected skin diseases.<sup>6</sup> In the study by Cornbleet et al<sup>6</sup> it was concluded that, based upon past experience, the administration of tetracycline (2 Gm. daily) with glucosamine was clinically more effective than the antibiotic alone. This effect would appear to be the logical consequence of higher antibiotic serum concentrations, as reported for glucosamine-potentiated tetracycline.<sup>1,7</sup>

The importance of initial treatment of upper respiratory infections in children, as has been stated in a previous report,<sup>5</sup> is often difficult because of the frequency of such infections and because etiological diagnosis is not always possible; hence, the antibiotic selected may not be specific for the organism. This problem has been compounded by the advent of staphylococcic infections resistant to many of the more commonly used agents.

Tetracycline with glucosamine was selected for this study on the basis of two important factors: (1) no broad-spectrum antibiotic is available for initial treat-

\* The trade name of Chas. Pfizer & Co. for glucosamine-potentiated tetracycline is Cosa-Tetracyn.

TABLE I  
*Sensitivity Tests and Results of Treatment*

[illegible]



TABLE II  
*Results of Treatment with Glucosamine-Potentiated Tetracycline*

Diagnosis	Number of cases	Response time, days	Results		
			Cured	No response	Not determined
Tonsillitis	24	2.5	20	3	1
Bronchitis	19	2.5	13	6	—
Pharyngitis	29	2.6	25	3	1
Croup	8	2.1	6	2	—
Pneumonitis	6	3.3	3	2	1
Tracheobronchitis	3	3.0	3	—	—
Otitis media	7	3.0	5	1	1
Cervical adenitis	4	3.5	2	2	—
	100	Av. 2.7	77	19	4

ment of all upper respiratory infections in children and infants; therefore, the broad-spectrum drug providing the most immediate and efficient antimicrobial attack would be the therapy of choice; and (2) the reported superiority of glucosamine-potentiated tetracycline over other preparations, its rapid achievement of higher antibiotic serum concentrations over tetracycline alone, as well as the possibility of its clinical superiority over tetracycline, were important considerations in this study.

The purpose of this study is to evaluate the clinical effectiveness of tetracycline with glucosamine given orally in the treatment of upper respiratory infections in children. Some conclusions must necessarily be inferred on the enhanced clinical effectiveness, if any, of glucosamine as an absorption-enhancing agent for tetracycline.

#### METHOD

One hundred febrile patients were treated for various upper respiratory infections (table I) or complications thereof. Ninety-nine patients, ages 6 months to 14 years old, and 1 patient, 20 years old, received glucosamine-potentiated tetracycline as oral suspension, reconstituted to 125 mg. per teaspoonful (5 ml.). In all cases, the drug was administered at four hour intervals. Seventy-eight patients received one teaspoonful of the syrup per dose. Of the remaining number, 13 patients, ages 6 months to 2 years old, received one-half teaspoonful of the preparation, 5 patients, ages 2½ to 14 years old, were given two teaspoonfuls, and 4 patients, ages 16 months to 9 years old received one teaspoonful in addition to 300,000 units of penicillin.

During the period of treatment with glucosamine plus tetracycline, no other antibiotics were administered, except as previously stated. Nonantibiotic preparations, such as eye drops and nose drops, were employed when indicated.

Eighty-seven of the 100 patients received glucosamine with tetracycline for one to three days. The remaining 13 patients were treated for the maximum period of four to six days.

Sensitivity tests were performed by means of the medicated disc method, utilizing penicillin, tetracycline, and chloramphenicol. Cultures of clinical specimens were taken from the nose and throat in all cases.

#### RESULTS

This study includes the results obtained on 100 patients, seen in private practice, who received tetracycline with glucosamine for upper respiratory infections or

coincident complications. Seventy-seven of the patients were cured, 75 within a period of one to three days.

Of the 23 patients remaining, 19 failed to respond to therapy until other antibiotic therapy was instituted, and results in 4 were "not determined," though 3 were cured and 1 became afebrile within 24 hours. The individual effect in 3 of these patients though cured, could not be ascertained since 300,000 units of penicillin had been administered in addition to tetracycline with glucosamine.

Several points of interest were noted upon conclusion of this investigation. In the 100 cases reported here, 28 patients with mixed infections, including staphylococci resistant to penicillin *in vitro*, were given glucosamine-potentiated tetracycline; 27 of these patients were cured, a recovery rate considerably higher than that of the entire group. But, more noteworthy, of 14 cases in which the organisms exhibited *in vitro* resistance to tetracycline, 6 patients, or slightly less than one-half the number of such patients, were cured, including 1 case of pharyngitis with stomatitis in which the gram-positive spores were also resistant to chloramphenicol and penicillin.

What are the implications behind this failure of correlation between laboratory susceptibility tests and clinical therapeutic response? While much of the evaluation rests with individual or combined studies including many more patients, the fact remains that of 100 patients, 6 per cent had tetracycline-resistant infections that were cured with glucosamine-potentiated tetracycline, an important margin of therapeutic success, particularly when an etiological diagnosis is not always possible. These results are comparable to findings reported by Stone et al<sup>3</sup> in which 7 of 8 cases resistant to tetracycline responded to treatment with glucosamine-potentiated tetracycline. It can only be concluded that the medical significance of such results, as related to higher antimicrobial serum levels attained more rapidly, is manifestly clear and certainly warrants further investigation.

Response to treatment with glucosamine-potentiated tetracycline was good in all cases. The period of treatment was one to six days; the average time required for cure was approximately 2.7 days (table II).

Glucosamine-potentiated tetracycline was extremely well tolerated; side effects were mild and transitory. The occurrence of a few loose stools in 16 patients did not necessitate interruption of therapy. One instance of vomiting occurred during the course of treatment; however, this effect could not be attributed specifically to the drug.

#### SUMMARY AND CONCLUSIONS

In summary it should be noted that with an antibiotic-enhancing agent combination, such as that which glucosamine-potentiated tetracycline represents, the exceptional clinical properties may not immediately be apparent in all cases. Obviously in treatment of the more common clinical entities, the patient in the usual situation will respond to one of several antibiotics. In addition, except under rather extraordinary circumstances, the response time alters very little. However, over some period of time, and perhaps several hundred patients, very often advantages of a given drug often come to the fore, in a sense by default. It appears that the effectiveness of glucosamine-potentiated tetracycline in the treatment of cases in which the microorganism is resistant to tetracycline is a noteworthy therapeutic advantage, particularly in pediatric cases in which treatment often must be initiated before results of sensitivity tests are available.

## BIBLIOGRAPHY

1. CARLOZZI, M.: Evaluation of antibiotic blood level enhancement factors, *AM & CT* 5:146, Feb., 1958.
2. SNELL, J. F., AND GARKUSCHA, R.: Radioactive oxytetracycline (Terramycin). III. Effect of glucosamine HCl on serum concentrations, *Proc. Soc. Exper. Biol. & Med.* 98:148, 1958.
3. STONE, M. L.; SEDLIS, A.; BAMFORD, J., AND BRADLEY, W.: Treatment of gynecological infections with glucosamine-potentiated tetracycline, *AM & CT* 5:322, 1958.
4. SHALOWITZ, M.: Treatment of upper respiratory infections with Cosa-Tetracyn, *Clin. Rev.* 1:25, Apr., 1958.
5. NATHAN, L. A.: Use of glucosamine-potentiated tetracycline in the treatment of upper respiratory infections in children, *Arch. Pediat.* 75:251, June, 1958.
6. CORNBLEET, T.; CHESROW, E., AND BARSKY, S.: Use of tetracycline with glucosamine in dermatology, *AM & CT* 5:328, 1958.
7. WELCH, H.; WRIGHT, W. W., AND STAFFA, A. W.: The effect of glucosamine on the absorption of tetracycline and oxytetracycline administered orally, *AM & CT* 5:52, 1958.

# Glucosamine-Potentiated Tetracycline in the Prophylaxis and Therapy of Surgical Infections

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Along with the great medical advances of the past generation in types of drugs—ataraxics, sulfonamides, steroids, antibiotics—there has been steady progress on a small, but no less important, scale in methods of administering drugs—special coatings, sustained-release preparations, and more recently, enhanced absorption.

Potentiating the effect of a drug by enhancing absorption is particularly desirable in administering drugs like antibiotics, for which it is important to produce adequate therapeutic levels quickly. Carlozzi<sup>1</sup> and Welch et al<sup>2</sup> recently demonstrated that preparations of tetracycline containing d-glucosamine, an amino sugar, produced definitely higher serum antibiotic concentrations than did commercial preparations of the antibiotics that did not contain glucosamine. In the past year I have had the opportunity to evaluate the efficacy of tetracycline potentiated with glucosamine under the less controllable conditions of clinical practice.

## PROCEDURE

Two groups of patients were treated in this evaluation of glucosamine-potentiated tetracycline: 28 men and women were treated for infections—abscess, fistula, felon, cyst, cellulitis and 24 men and women were given this antibiotic prophylactically to prevent postoperative wound infection. Patients ranged in age from 15 to 70 years; the average age was 45.2 years.

Four times daily each patient received 1 capsule containing 250 mg. of tetracycline and 250 mg. of glucosamine.\* The preparation was administered for 2 to 51 days; the average duration of therapy was 8.9 days. No other antimicrobial agents were administered during the period of therapy; however, appropriate concurrent treatment such as wet dressings, drainage, and irrigation was used whenever indicated.

Patients were observed carefully for response to medication. Nineteen of the 28 patients who suffered from infection received sensitivity tests of the pathogenic organism, and multiple serial cultures of the organism were made on several media during the study.

## RESULTS

*Antibacterial.* All 28 patients who received the antibiotic to combat infection were cured within 2 to 20 days. A tabulation of patient response and type and sensitivity of the pathogenic organism (table I) is in accordance with the well-known broad spectrum of activity of tetracycline and emphasizes that in vitro sensitivity tests do not always accurately predict the in vivo activity of an antibiotic; e.g., patient 2 (table I) was found to be free of infection from *Staphylococcus aureus* after three days of treatment with glucosamine-potentiated tetracycline, al-

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\* The trade name of Chas. Pfizer & Co. for a combination of tetracycline and glucosamine is Cosa-Tetracycln.

TABLE I  
Efficacy of Glucosamine-Potentiated Tetracycline

Patient	Pathogen	Susceptibility of pathogen*							Patient response to tetracycline
		Tetracycline	Penicillin	Streptomycin	Erythromycin	Oleandomycin	Novobiocin	Chloramphenicol	
1	<i>Staph. aureus</i>	+	—	+	—	—	+	—	Cure
2	<i>Staph. aureus</i>	—	—	—	—	—	+	—	Cure
3	<i>Staph. aureus</i>	—	—	—	+	—	—	+	Cure
4	<i>Staph. aureus</i>	+	—	—	+	+	+	+	Cure
5	<i>Staph. aureus</i>	+	+	—	+	—	+	—	Cure
6	<i>Staph. aureus</i>	+	—	+	+	—	+	+	Cure
7	<i>Staph. aureus</i>	—	+	—	—	—	+	—	Cure
8	<i>Staph. albus</i>	—	—	—	—	—	+	—	Cure
9	<i>Staph. albus</i>	+	+	+	—	+	+	—	Cure
10	<i>Staph. albus</i>	+	+	+	+	+	—	—	Cure
11	<i>Staph. albus</i>	+	+	+	+	+	+	+	Cure
12	alpha <i>Streptococcus</i>	—	+	—	—	—	+	+	Cure
13	alpha <i>Streptococcus</i>	+	—	+	—	—	—	+	Cure
14	alpha <i>Streptococcus</i>	—	—	—	—	—	—	+	Cure
15	<i>Escherichia</i> (gram-negative)	+	—	+	—	—	—	+	Cure
16	<i>Escherichia</i> (gram-negative)	—	—	+	—	—	—	+	Cure
17	<i>Escherichia</i> (gram-negative)	+	—	+	—	—	—	+	Cure
18	Streptococcal alpha diphtheroids	—	+	+	+	+	—	—	Cure
19	<i>Staphylococcus</i> (unidentified)	—	—	—	—	—	+	—	Cure

\* — = resistant; + = sensitive.

though the organism was reported resistant to the unpotentiated antibiotic in laboratory tests.

*Prophylactic Use.* There is no way to demonstrate prophylactic efficacy of the preparation except by the negative evidence that none of the patients treated prophylactically in this study developed infections.

*Untoward Reactions.* Four of the 52 patients had untoward reactions to therapy. In 2 of these patients the reactions were mild and transitory (diarrhea, 1 patient, and nausea, 1 patient); in 2 patients the reactions were more severe and therapy was discontinued, although the antibiotic was therapeutically effective (diarrhea, 1 patient, and both vaginitis and proctitis, 1 patient). This incidence of untoward reactions is within the range previously reported for tetracycline.

#### DISCUSSION

The simple statement of cures or lack of infection, the objective quantitative data of the study, must be evaluated in light of the subjective qualitative results. The purpose of the antibiotic therapy was to prevent or eliminate infection. For both purposes, tetracycline potentiated with glucosamine was particularly effective. The time spent by patients in the hospital was less than would otherwise be expected, and because satisfactory therapeutic tissue levels of the antibiotic could be achieved rapidly and maintained constantly with oral administration of the preparation, many patients who might have had to be hospitalized could be treated as outpatients. The resultant reduction in duration and complexity of surgical treatment was especially gratifying.

#### SUMMARY

Tetracycline with glucosamine was administered to 52 adults either as a prophylactic agent before and after surgery or as an antimicrobial agent to combat infection. In both groups, all patients responded satisfactorily.

#### BIBLIOGRAPHY

1. CARLOZZI, M.: Evaluation of antibiotic blood level enhancement factors, *AM & CT* 5:146, 1958.
2. WELCH, H.; WRIGHT, W. W., AND STAFFA, A. W.: The effect of glucosamine on the absorption of tetracycline and oxytetracycline administered orally, *AM & CT* 5:52, 1958.

# The Effect of Triamcinolone, Prednisolone, and Hydrocortisone on Dosage Requirements of Tetracycline for Control of a *Streptococcus* Infection in Mice

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Jawetz<sup>1</sup> and Foley<sup>2</sup> have shown that treatment with large dosages of cortisone increases the therapeutic dosage requirements of broad-spectrum antibiotics for experimental infections. Foley et al<sup>3</sup> have also shown that cortisone, hydrocortisone, prednisolone, and prednisone are of equal potency in this respect. The present report is concerned with the rating of triamcinolone for similar activity. In this work triamcinolone (9 $\alpha$ -fluoro-11 $\beta$ -16 $\alpha$ , 17 $\alpha$ , 21-tetrahydroxy-1,4-pregnadiene-3,20-dione) was compared with hydrocortisone and prednisolone ( $\Delta^1$  hydrocortisone) in regard to its ability to change the tetracycline requirements for controlling a *Streptococcus* infection in mice.

## EXPERIMENTAL

The animals used in this study were male albino mice, strain CF1, supplied by Carworth Farms. Mice, 6 to 8 weeks of age, weighing 18 to 22 Gm., were used in unit test groups of 10 mice/cage.

The infection was produced by intra-abdominal injection of 0.5 ml. of a 10<sup>-5</sup> broth dilution of a five hour blood broth culture of *Streptococcus pyogenes*, beta-hemolytic strain C203. The infecting doses contained 1500  $\pm$  200 viable units as determined by plate counts. The mortality rate for untreated infected controls was 98 per cent (<sup>98</sup>/<sub>100</sub>), with an average survival time of 1.1 days.

The steroids were suspended in carboxymethylcellulose and the tetracycline hydrochloride dissolved in 0.2 per cent aqueous agar in amounts that would produce the desired dose in 0.5 ml. of the preparation. Immediately after infection, each mouse was injected subcutaneously with a steroid suspension or the suspending vehicle. One hour later each mouse was injected subcutaneously with an antibiotic preparation or the diluent. The mice were observed for 14 days after infection.

The quantitative measurements of the antistreptococcal efficacy of tetracycline alone, or in combination with a steroid, were made by pooling the results of replicate tests on each dosage level and applying the procedures of Litchfield and Wilcoxon<sup>4</sup> for evaluating dosage-effect experiments. The association between dosage of steroid and median effective dosage (ED<sub>50</sub>) of tetracycline was established by the application of the method of least squares. Relative potency ratings of the steroids were determined by comparing the steroid dosages required for equivalent tetracycline ED<sub>50</sub> as indicated by the least squares lines.

## RESULTS

The results of a preliminary test to determine the effect of steroid doses of 80, 20, or 5 mg./Kg. administered to uninfected mice by a single subcutaneous injection showed the effect to be one of interference with weight gain. A dose of 80 mg./Kg.

TABLE I

*Hydrocortisone Versus Prednisolone:  
Effect on Tetracycline Requirements for Control of a Streptococcus Infection in Mice  
(Single Subcutaneous Injection)*

Tetra- cycline, mg./Kg.	Carboxymethyl- cellulose control		Hydrocortisone, mg./Kg.						Prednisolone, mg./Kg.					
			80		20		5		80		20		5	
	S.R.*	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect
320			20/20	100	27/30	90			18/20	90	28/29	97		
160	25/30	83	22/30	73	28/39	72	25/30	83	13/30	43	30/40	75	25/30	83
80	19/29	66	10/30	33	20/39	51	20/30	67	11/30	37	16/40	40	20/30	67
40	17/30	57	6/30	20	15/39	38	11/29	38	9/30	30	7/39	18	10/30	33
20	11/30	37	0/10	0	3/10	30	7/30	23	0/10	0	2/10	20	10/30	33

\* S.R. = survival ratio. Number of animals alive on fourteenth day after infection/number tested.

of triamcinolone produced a weight loss for a few days, while an equal dose of hydrocortisone or prednisolone merely inhibited gain in weight. The 5 mg./Kg. dose level of the three steroids was relatively without effect.

The results of four tests showed that carboxymethylcellulose, the suspending medium for the steroids, had no effect on the tetracycline requirements to control the *Streptococcus* infection. Therefore, the groups of mice treated with steroid and tetracycline were compared with groups treated with carboxymethylcellulose and tetracycline.

The pooled results of tests in which hydrocortisone and prednisolone were compared for effect on tetracycline response are shown in table I. Results of the comparison of hydrocortisone and triamcinolone are shown in table II. It is evident that equivalent tetracycline doses afforded greater protection when administered to mice without steroid treatment. The median effective tetracycline doses and the relative potency of the antibiotic in each combination with a steroid were calculated from these results (table III). These data show that less tetracycline was needed for an ED<sub>50</sub> when administered alone than when administered with any of the three steroids. The larger doses of steroid increased significantly the tetracycline requirement for equivalent response against the infection.

A statistical analysis of these data indicated that the tetracycline ED<sub>50</sub> could be associated with the steroid doses by least squares lines with a common slope. The

TABLE II

*Hydrocortisone Versus Prednisolone:  
Effect on Tetracycline Requirements for Control of a Streptococcus Infection in Mice  
(Single Subcutaneous Injection)*

Tetra- cycline, mg./Kg.	Carboxymethyl- cellulose control		Hydrocortisone, mg./Kg.						Triamcinolone, mg./Kg.					
			80		20		5		80		20		5	
	S.R.*	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect
320			20/20	100	27/30	90			17/20	85	27/30	90	10/10	100
160	26/30	87	21/30	70	27/39	69	23/30	77	15/30	50	25/40	63	23/30	77
80	19/30	63	11/30	36	15/39	38	17/30	57	6/30	20	13/40	33	17/30	57
40	14/30	47	7/30	23	11/39	28	11/30	37	0/30	0	8/40	20	10/30	30
20	11/30	37	0/10	0	3/10	30	7/30	23	0/10	0	0/10	0	5/20	25

\* S.R. = survival ratio. Number of animals alive on fourteenth day after infection/number tested.

TABLE III

Comparison of Median Effective Dosages of Tetracycline for a *Streptococcus*  
Infection in Mice Treated with and without Steroids  
(Dosage Effect Curve Parameters)

Steroid	mg./Kg.	Tetracycline		
		ED <sub>50</sub> , mg./Kg.	Slope function*	Relative activity
<i>Comparison no. 1 (see table I)</i>				
None	Carboxymethyl-cellulose control	36 ( 25– 53)	4.7 (2.0–11 )	1.00
Hydrocortisone	80	110 ( 81–140)	2.6 (1.9– 3.5)	0.33 (0.21–0.52)
	20	66 ( 48– 90)	3.7 (2.3– 5.9)	0.55 (0.34–0.90)
	5	51 ( 36– 71)	3.1 (1.8– 5.3)	0.71 (0.43–1.18)
Prednisolone	80	110 ( 70–160)	4.2 (2.3– 7.8)	0.33 (0.19–0.57)
	20	86 ( 69–110)	2.1 (1.7– 2.7)	0.42 (0.27–0.65)
	5	48 ( 35– 66)	3.5 (2.3– 5.4)	0.75 (0.46–1.24)
<i>Comparison no. 2 (see table II)</i>				
None	Carboxymethyl-cellulose control	38 ( 26– 55)	4.5 (2.0–10 )	1.00
Hydrocortisone	80	100 ( 73–140)	3.0 (2.0– 4.4)	0.38 (0.23–0.62)
	20	89 ( 68–120)	2.8 (1.9– 4.1)	0.43 (0.27–0.68)
	5	60 ( 43– 84)	4.0 (2.0– 8.0)	0.63 (0.26–1.04)
Triamcinolone	80	150 (120–200)	2.1 (1.7– 2.6)	0.25 (0.16–0.39)
	20	110 ( 83–130)	2.6 (2.0– 3.3)	0.35 (0.23–0.54)
	5	62 ( 44– 88)	3.7 (2.3– 6.0)	0.61 (0.41–0.90)

\* Slope function =  $\frac{ED_{50}/ED_{10} + ED_{50}/ED_{90}}{2}$ . Figures in parentheses indicate 95 per cent confidence limits.

formulas for these lines were calculated to be as follows: hydrocortisone:  $\log y = 1.5313 + 0.270 (\log x)$ ; prednisolone:  $\log y = 1.5347 + 0.270 (\log x)$ ; triamcinolone:  $\log y = 1.6517 + 0.270 (\log x)$ . These lines are shown in figure 1.

It is apparent that triamcinolone increased to a greater extent the tetracycline requirement for a median effect against the infection than did hydrocortisone or prednisolone. The tetracycline ED<sub>50</sub> associated with the various steroid doses was redetermined on the basis of these lines (table IV). An analysis of variance showed the triamcinolone effect to be significantly greater than that of either hydrocortisone or prednisolone. The latter two steroids were equal in effect.

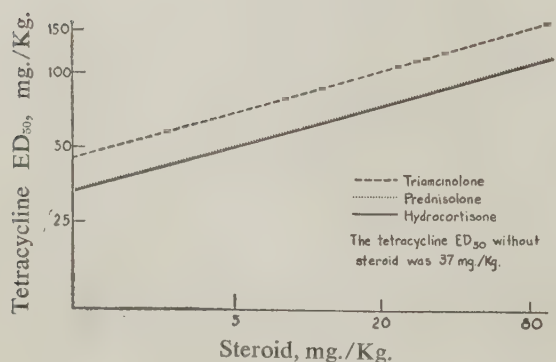


FIG. 1. The effect of steroid treatment of mice on the tetracycline ED<sub>50</sub> against the *Streptococcus* C203 infection is shown.

TABLE IV

*Tetracycline ED<sub>50</sub> for Streptococcus-Infected Mice Treated with Steroids  
(Based on Least Squares Lines)*

Steroid dose, mg./Kg.	Tetracycline ED <sub>50</sub> , mg./Kg.		
	Hydrocortisone	Prednisolone	Triamcinolone
80	111	112	146
20	76	77	100
5	52	53	68

The tetracycline ED<sub>50</sub> for mice without steroid treatment was 37 mg./Kg.

## DISCUSSION

This study shows that hydrocortisone, prednisolone, and triamcinolone were qualitatively alike with respect to increasing the dose of tetracycline necessary to produce equivalent protection against a standardized *Streptococcus* infection in mice. The tetracycline ED<sub>50</sub> was significantly higher when administered to mice treated with the higher doses of steroids. Hydrocortisone and prednisolone were found to be equal in this effect, which confirms the work of Foley et al.<sup>3</sup> Triamcinolone, however, was 2.7 times more potent because 6.6 mg./Kg. of this steroid, in comparison to 18 mg./Kg. of each of the other two steroids, was calculated as the dosage associated with a twofold increase in tetracycline ED<sub>50</sub> (table V).

Triamcinolone is physiologically more active than either hydrocortisone or prednisolone. It was 3 to 12 times more potent than prednisolone and 10 to 40 times more active than hydrocortisone in liver glycogen deposition tests; it proved to be 5 to 10 times more active than hydrocortisone acetate in an anti-inflammatory assay; it was more active than hydrocortisone or prednisolone in maintaining the life of adrenalectomized immature male rats.<sup>5</sup> In the present study, a preliminary test showed that triamcinolone was more potent than hydrocortisone or prednisolone with respect to interfering with the normal gain in weight of mice. The greater potency of triamcinolone in terms of increasing the amount of tetracycline needed for protection of mice infected with streptococci is another indication that it is a more active drug physiologically than either hydrocortisone or prednisolone.

TABLE V

*Relative Potency Ratings of Hydrocortisone,  
Prednisolone, and Triamcinolone for Increasing Tetracycline  
Requirements for Control of a Streptococcus Infection in Mice*

Steroid	Steroid dosage associated with tetracycline ED <sub>50</sub> of 74 mg./Kg.*	Relative steroid potency
Hydrocortisone	18.0	1.0
Prednisolone	18.0	1.0
Triamcinolone	6.6	2.7

\* Since the response lines were parallel, this tetracycline ED<sub>50</sub> was arbitrarily chosen to illustrate estimated relative dosages of steroids required for equal effect. It represents a twofold increase in tetracycline ED<sub>50</sub> compared to the control tetracycline ED<sub>50</sub> (37 mg./Kg.) without steroid.

Triamcinolone was compared with hydrocortisone and prednisolone with respect to its ability to change the tetracycline requirements for controlling a *Streptococcus* infection in mice. The three steroids were qualitatively alike in that treatment with high doses produced a significant increase in tetracycline requirements. Triamcinolone was approximately three times more potent than either hydrocortisone or prednisolone in producing this effect.

## ACKNOWLEDGMENTS

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## BIBLIOGRAPHY

1. JAWETZ, E.: Effect of cortisone on therapeutic efficacy of antibiotics in experimental infection, *A.M.A. Arch. Int. Med.* 93:850-862, 1954.
2. FOLEY, E. J.: Therapeutic effect of chlortetracycline and oxytetracycline in immunized mice treated with cortisone, *Antib. & Chemo.* 5:1-5, 1955.
3. FOLEY, E. J.; MORGAN, W. A., AND GRECO, G.: Effect of prednisone and prednisolone on *Streptococcus* infections in mice treated with chlortetracycline, *Antib. & Chemo.* 7:65-69, 1957.
4. LITCHFIELD, J. T., JR., AND WILCOXON, F.: A simplified method of evaluating dose-effect experiments, *J. Pharmacol. & Exper. Therap.* 95:99-113, 1949.
5. PERRINE, J. W.; BORTLE, L.; HEYDER, E.; PARTRIDGE, R.; ROSS, E. K., AND RINGLER, I.: Adrenal corticoid activities of 9 $\alpha$ -fluoro-11 $\beta$ -16 $\alpha$ , 17 $\alpha$ , 21-tetrahydroxy-1,4-pregnadiene-3, 20-dione (triamcinolone). In press.

# Polyphloreitin Phosphate Complexes of Tetracycline and Related Compounds

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In 1953, Diczfalussy et al<sup>1</sup> described the preparation of a series of high molecular weight, synthetic, polymeric esters of phosphoric acid and polyphenols. These compounds were found to be the most potent inhibitors of alkaline phosphatase known and, in addition, had a strong inhibiting effect on both hyaluronidase and urease. One of the most active compounds was polyphloreitin phosphate, which was obtained by the phosphorylation of phloreitin, arresting the spontaneously occurring polymerization at a molecular weight of approximately 15,000, and isolation of the polymerized product. In animal, clinical, and metabolic studies,<sup>2-5</sup> it was shown that polyphloreitin phosphate exerted a remarkable enhancing effect on ACTH and delayed the action of insulin.<sup>6</sup>

Ever since the introduction of the tetracyclines, the search for a dosage form of these antibiotics has been under way that would result in high and sustained blood levels with either oral or parenteral administration and that would be well tolerated on intramuscular injection. Our attention was therefore directed to polyphloreitin phosphate, which, due to its enzyme-inhibiting effect, might enhance the efficacy of tetracycline when given in conjunction with it.

## MATERIALS AND METHODS

When aqueous solutions of tetracycline hydrochloride are reacted with dilute aqueous solutions of polyphloreitin phosphate, immediate precipitates form. Isolated, they are yellow amorphous solids, which possess a relatively constant composition when assayed spectrophotometrically for their tetracycline contents. Irrespective of the fact that the reacting solutions may contain a large excess of either one or the other reactant, the final composition remains constant. For example, when aliquots of a solution, each containing 200 mg. tetracycline hydrochloride, were reacted with solutions of polyphloreitin phosphate, containing from 40 to 400 mg. each, the washed and dried precipitates contained between 50.6 and 54.1 per cent tetracycline.

The equivalence point was determined by the following method. The clear supernatants of the precipitates were divided into two portions each. To one there was added a volume of the tetracycline solution, to the other portion a volume of the polyphloreitin phosphate solution. The supernatant that contained the smallest excess of either component yielded the least turbidity in both portions, as determined turbidimetrically. The ratio of the reactants at this point, designated as the "isophane" ratio, was taken as the stoichiometric equivalence point and occurred at approximately one part of tetracycline to 1.1 parts of polyphloreitin phosphate. The isophane ratio may vary, according to the batch of polyphloreitin phosphate used, depending on its purity, degree of polymerization, and available functional groups.

A typical preparation of tetracycline-polyphloreitin phosphate complex then proceeded as follows: 22.0 Gm. polyphloreitin phosphate of 76.7 per cent purity were dissolved in 540 ml. water. The solution was clarified with the aid of Supercel and was added slowly, with rapid agitation, to a freshly prepared solution of 20.0 Gm.

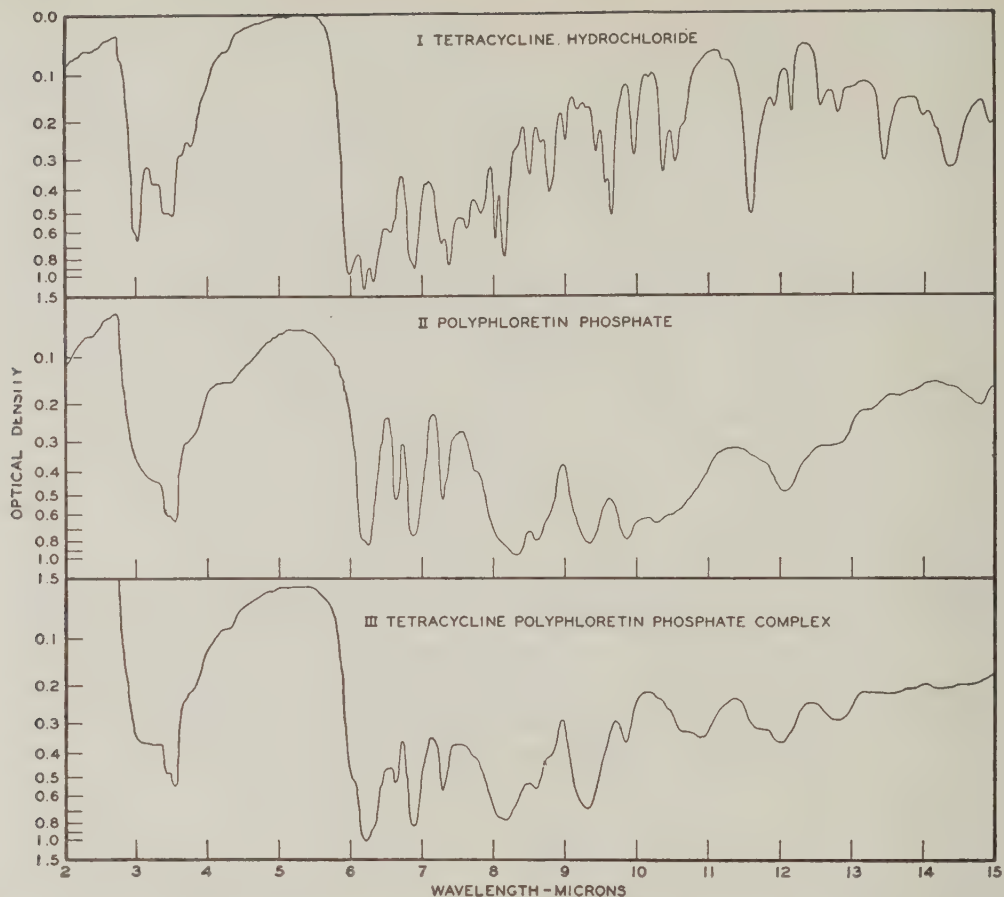


FIG. 1. The infrared spectra of tetracycline hydrochloride, polyphloreitin phosphate, and tetracycline polyphloreitin phosphate complex are shown.

tetracycline hydrochloride in 190 ml. water. The suspension thus formed was stored in the refrigerator for a number of hours; the precipitate was then filtered off and freed of mother liquor. The solids were slurried twice with a minimum amount of water and washed until free of chloride. After final washes with a small

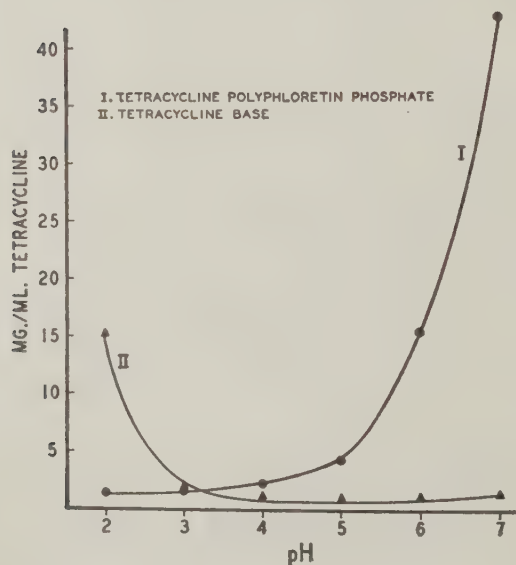


FIG. 2. The solubility curves of tetracycline polyphloreitin phosphate and tetracycline base are given.

TABLE I  
Results of the Intravenous and Intraperitoneal Toxicity Tests

	Average LD <sub>50</sub> mg./Kg. $\pm$ S.E.		Average estimated LD <sub>2</sub> mg./Kg.	
	Complex	Tetracycline equivalent	Complex	Tetracycline equivalent
Acute intravenous toxicity	290 $\pm$ 12	144	218	108
Acute intraperitoneal toxicity	410 $\pm$ 39	203	210	104

volume of acetone and ether, the solid was dried to constant weight at 40 C. over phosphorus pentoxide in vacuo. No further purification steps were carried out. A finely divided yellow powder 28.2 Gm., was obtained, which represents approximately 80 per cent of the theoretical yield.

The elemental analysis showed nitrogen = 3.12 per cent and phosphorus = 5.26 per cent on the dry basis. These values, when taking 6.3 per cent for nitrogen in tetracycline and 10.30 per cent for phosphorus found in the polyphloreitin phosphate employed, calculate to a tetracycline content of 49.6 per cent and polyphloreitin phosphate, 51.0 per cent.

The ultraviolet spectrum shows the tetracycline peak at 380  $m\mu$  when dissolved in N/10 sodium hydroxide. At this wavelength,  $E_{1\text{cm.}}^{1\%} = 180.8$ , which, when considering the  $E_{1\text{cm.}}^{1\%}$  value = 360.6 of the tetracycline hydrochloride used, calculates to a tetracycline equivalent of 502 u./mg.

The microbiological assay, using *Micrococcus pyogenes* var. *aureus* as test organism, showed a tetracycline equivalent of 490 u./mg., when calculated on the dry basis.

The infrared spectra of tetracycline hydrochloride, polyphloreitin phosphate, and tetracycline polyphloreitin phosphate complex are shown in figure 1.

#### SOLUBILITY STUDIES

The solubility properties of the complex follow the pattern of polyphloreitin phosphate, rather than that of tetracycline. Under acidic conditions, the solubility is extremely limited, increasing rapidly with a rising pH and reaching a high order of magnitude at neutrality. This constitutes a marked contrast to tetracycline, which possesses just the opposite properties, passing from a very high solubility at a low pH to a very limited one at or near neutrality. The solubility of the complex was determined by shaking an excess of the solid in M/7.5 sodium phosphate buffer solution, adjusted to the requisite pH values, until equilibrium was reached, centrifuging and filtering the supernatant, and determining the concentration of complex in solution spectrophotometrically. The solubility curves of the complex and tetracycline base are shown in figure 2.

#### TOXICOLOGICAL STUDIES

For acute intravenous toxicity studies, the tetracycline polyphloreitin phosphate complex was prepared as a 3 per cent solution in a M/10 sodium phosphate buffer at pH 6.5. A total of 35 male mice were injected intravenously at three or more logarithmically spaced dosage levels, and the animals were observed for 10 to 11 days. Any deaths occurring in this period were included in the calculation of the average LD<sub>50</sub> and the average estimated LD<sub>2</sub>. For determination of the intraperitoneal toxicity, the sample was prepared as a 5 per cent suspension in 0.25 per cent agar and injected intraperitoneally into a total of 30 female mice. The re-

TABLE II  
*Relative Tetracycline Activity on Oral Administration*

Hours after dose			
1	2	4	7
0.34 ± 1.2	0.53 ± 0.3	0.78 ± 0.3	1.01 ± 0.8

sults of these tests are shown in table I. The results indicate that the presence of polyphloreitin phosphate does not materially affect the toxicity of tetracycline, which has been reported<sup>7</sup> as  $162 \pm 14$  mg./Kg. for the intravenous and  $190 \pm 32$  mg./Kg. for the intraperitoneal LD<sub>50</sub>.

In exploratory experiments for the determination of the degree of tissue irritation upon intramuscular injection, three preparations were compared: (1) tetracycline polyphloreitin phosphate complex, buffered to yield a solution at pH 6.5 containing 100 mg. tetracycline equivalent upon constitution with 2 ml. water for injection, U. S. P.; (2) tetracycline polyphloreitin phosphate complex, unbuffered, yielding a suspension at a corresponding tetracycline concentration as in (1); and (3) tetracycline hydrochloride (Steclin\*) in the form of the regular intramuscular product blended with ascorbic acid, magnesium chloride, and procaine hydrochloride, and having a pH of 2.2. Single 0.25 ml. injections of the test samples were made into the vastus lateralis muscles of 11 rabbits. Six of the animals were sacrificed after two days and the remainder after seven days, the injected muscles were excised and examined grossly for local irritation. After two days, no significant differences of the three formulations were apparent, however, after seven days, the lesions produced by sample II showed a considerably greater regression than those of I and III.

#### BLOOD LEVEL STUDIES

Preliminary studies of the blood serum levels of tetracycline activity produced following oral and intramuscular administration of tetracycline polyphloreitin phosphate as compared to tetracycline hydrochloride and phosphate were made in dogs.

The *Bacillus cereus* agar diffusion assay was used to evaluate the tetracycline activity following the distribution of each serum sample into two or three cups per plate, duplicated over four plates.

*Oral.* Crossover studies were carried out in 8 fasted dogs to show the average levels of tetracycline activity produced in the blood serum at 1, 2, and 4 hours following oral administrations equivalent to 10 mg. tetracycline activity per Kg. of tetracycline polyphloreitin phosphate and tetracycline hydrochloride. The inhibition zones produced by the tetracycline in the serum samples were calculated in terms of the tetracycline hydrochloride as 1.0 and the values obtained are shown in table II. These levels indicate that while tetracycline polyphloreitin phosphate, on oral administration, initially exhibits lower blood concentrations, at later comparative hours they approach those levels attained by tetracycline hydrochloride.

*Intramuscular.* For the intramuscular studies, 12 dogs were employed in a crossover pattern for the determination of serum levels upon intramuscular injection of unbuffered aqueous suspensions of tetracycline polyphloreitin phosphate and tetracycline phosphate complex.† The administered dose in each case was equivalent to

\* The trade name of E. R. Squibb & Sons, Division of Olin Mathieson Chemical Corp., for tetracycline hydrochloride is Steclin.

† The trade name of E. R. Squibb & Sons, Division of Olin Mathieson Chemical Corp., for tetracycline phosphate complex is Sumycin.

TABLE III  
*Blood Serum Levels on Intramuscular Injection*

	Hours after dosage Activity per ml. serum $\pm$ S.E.				
	1	2	4	7	24
Tetracycline phosphate	1.08 $\pm$ 0.18	1.13 $\pm$ 0.23	1.10 $\pm$ 0.19	1.46 $\pm$ 0.07	0.46 $\pm$ 0.05
Tetracycline polyphlorein phosphate	0.73 $\pm$ 0.06	1.02 $\pm$ 0.07	1.09 $\pm$ 0.05	1.24 $\pm$ 0.10	0.43 $\pm$ 0.03

5 mg. tetracycline activity per Kg. Serum samples were assayed at 1, 2, 4, 7, and 24 hours after the injection. The average tetracycline blood serum levels obtained are summarized in table III.

This indicates that upon intramuscular injection suspensions of tetracycline phosphate and tetracycline polyphlorein phosphate give rise to similar blood serum levels.

#### COMPLEXES FORMED WITH RELATED ANTIBIOTICS

Polyphlorein phosphate reacts with chlortetracycline and oxytetracyclines in a similar manner as it does with tetracycline. Complexes are readily formed upon the interaction of aqueous solutions of the compounds and their physical properties are similar to those reported here for the tetracycline complex.

#### SUMMARY

A novel tetracycline polyphlorein phosphate complex has been described with details as to its method of preparation and some of its chemical, physical and pharmacological properties.

#### ACKNOWLEDGMENTS

The authors wish to express their appreciation to J. Alicino for elemental analyses, to Dr. Rhoda Stasiak and Charles Lendzian for the ultraviolet and to Joseph Kulesza, Milton Nasveschuk, Herbert Stander, and Dr. R. Moe for the toxicological studies and blood level determinations.

#### BIBLIOGRAPHY

1. DICZFALUSY, E.; FERNDE, O.; FEX, H.; HOEGBERG, B.; LINDEROT, T., AND ROSENBERG, T.: Synthetic high molecular weight enzyme inhibitors. I. Polymeric phosphates of phlorein and related compounds, *Acta chem. Scandinav.* 7:913-920, 1953.
2. HAMBURGER, C.: The effect of polyphlorein phosphate on the ascorbic acid depleting activity of ACTH, *Acta endocrinol.* 11:282-296, 1952.
3. FISCHER, F.; FRIIS-HANSEN, B.; GEORG, J.; HASTRUP, B.; KALBOCK, K.; SPRECHLER, M.; WARMING-LARSEN, A., AND WULFF, H.: Effect of ACTH in polyphlorein phosphate solution (clinical and metabolic studies), *Acta endocrinol.* 13:293-305, 1953.
4. WEIDMANN, H.: Enhancement of the ascorbic acid depleting activity of ACTH by polyphlorein phosphate, *Acta endocrinol.* 13:287-292, 1953.
5. COHEN, H.; FREEDMAN, H. H.; KLEINBERG, W.; EISLER, N., AND MARTIN, G. J.: Enhancement of adrenocorticotrophic activity, *Proc.* 82:749-751, 1953.
6. WEIDMANN, H.: The effect of polyphlorein phosphate on the absorption of insulin, *Acta endocrinol.* 18:81-86, 1955.
7. ENGLISH, A. R.; P'AN, S. Y.; MCBRIDE, T. J.; GARDOCKI, J. F.; VAN HALSEMA, G., AND WRIGHT, W. A.: Tetracycline—microbiologic, pharmacologic, and clinical evaluation. In: *Antibiotics Annual 1953-1954*, New York, Medical Encyclopedia, Inc., 1954, p. 70.

# Laboratory and Clinical Studies with a New, Preconstituted, Intramuscular Solution of Oxytetracycline

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Since their introduction, all intramuscular dosage forms of the tetracycline antibiotics available to the physician have consisted of dry powder formulations that have to be reconstituted with water prior to administration. Oxytetracycline intramuscular, for example, which has been the subject of several publications from these laboratories,<sup>1,2,3</sup> consists of an admixture of the antibiotic hydrochloride with magnesium chloride and procaine hydrochloride. A preconstituted intramuscular solution of oxytetracycline has now been developed and evaluated clinically with favorable results.

Heretofore, the poor solubility of the tetracyclines in neutral and weakly acidic or basic pH regions, as well as the marked instability of strongly acid or alkaline solutions, have thwarted attempts to devise a preconstituted product suitable for intramuscular therapy. Thus it is recommended that the oxytetracycline\* and tetracycline† dosage forms be utilized within 24 hours following their reconstitution. On the other hand, it has been noted that basic salts of certain metal chelates of oxytetracycline are readily soluble in aqueous organic vehicles and that resulting solutions exhibit extraordinary stability when protected from atmospheric oxygen. Salts of the metal chelates may be synthesized and then dissolved in a suitable vehicle, or the antibiotic derivative may be prepared directly in situ. After considerable chemical and pharmacological experimentation, an ethanolammonium magnesium oxytetracycline preparation in 50 per cent aqueous *N,N*-dimethylacetamide was chosen as a suitable candidate for clinical evaluation. Since there existed the possibility that clinical investigation might indicate the desirability of incorporating a local anesthetic in the solution, formulations containing 2 per cent lidocaine were also prepared. Lidocaine proved to be the anesthetic of choice not only because of its excellent anesthetic properties but also because of its chemical stability in aqueous *N,N*-dimethylacetamide at pH 8.5, the pH of the oxytetracycline solution.

## LABORATORY STUDIES

*Stability Data.* Summarized in table I are biological stability data for several typical lots of the new oxytetracycline intramuscular solution contained in glass ampoules that were flushed with nitrogen before sealing. In view of these accelerated stability studies, it is apparent that complete bio-potency retention may be anticipated for periods in excess of one year.

*Animal Serum Level Data.* Having demonstrated the practicality of a preconstituted oxytetracycline solution from a stability standpoint, attention was focused upon the magnitude and duration of the oxytetracycline serum levels and the degree of irritation produced by the injection of the solution in animals.

A group of rabbits was injected intramuscularly with the experimental solution, while another group received an equivalent dose of an aqueous solution of the

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\* The trade name of Chas. Pfizer & Co. for oxytetracycline is Terramycin.

† The trade name of Chas. Pfizer & Co. for tetracycline is Tetracyn.

TABLE I  
*Biological Activity of Oxytetracycline Intramuscular Solution ( $\mu\text{g./ml.}$ ) Versus  
Klebsiella pneumoniae*

Lot no.	Initial activity	Temp., C.	Activity after initial				
			3 wk.	6 wk.	12 wk.	6 mo.	1 yr.
2352-270	50.3	50	50.0	49.8	44.2	37.8	50.3
		37		51.1	47.9	42.4	
		25					
2785-62-2	46.9	50	39.0	44.4	41.5		44.1
		37		41.8	44.7	46.0	
		25				47.5	
2785-119	50.5	50	48.9	42.6	48.6		
		37		48.1		49.1	
		25				53.1	
4026-56-3*	51.0	50	51.7	51.1	48.4		
4026-63-3*	49.7	50	52.1	50.4	51.1		
4026-77-3*	50.7	50	51.6	55.6	49.0		

\* These lots contained 2 per cent lidocaine.

commercially available dry powder intramuscular dosage form of oxytetracycline. The results of these experiments are shown for two lots of solution in table II. The ratios listed in the table indicate the number of animals showing a measurable level to the total number bled at a given time. From the rabbit studies, it may be concluded that oxytetracycline intramuscular solution produces the same serum level pattern as that resulting from an aqueous solution of the dry powder formulation.

*Intramuscular Irritation Studies in Animals.* Rabbits were given single and repeated injections of 1 ml. of solution containing 50 mg. of antibiotic activity. This dose amounted to approximately 25 mg./Kg., which is about 16 times the dose usually administered to human beings. In typical experiments the following observations were made.

**SINGLE INJECTION.** Rabbits were injected in each of their gluteus maximus muscles with 1 ml. of solution containing 50 mg. of oxytetracycline. When the animals were sacrificed after 24 hours, the muscles showed no evidence of irritation in the fresh state and only trace amounts of residual drug could be observed. After fixation in formalin, small lesions were noted in some muscles, but these were minimal and comparable to those noted in control animals receiving the dry powder formulation.<sup>2</sup>

**REPEATED INJECTION.** Rabbits were sacrificed after receiving three daily injections

TABLE II  
*Oxytetracycline Intramuscular Solution Animal Serum Levels*

Lot no.	Serum levels, $\mu\text{g./ml.}$ , at various times, hr.							
	0	1	3	5	7	18	24	30
2785-41*	0	2.65	2.68	2.33	1.50	0.883	0.183	0.136
		7/7	7/7	7/7	7/7	6/6	6/6	4/5
Control†	0	3.05	2.59	1.88	1.34	0.245	0.179	0.172
		5/5	5/5	5/5	5/5	2/2	5/5	5/5
2532-284*	0	2.96	2.28	1.36	1.11	0.368	0.098	0.088
		8/8	8/8	8/8	8/8	5/5	3/8	2/7
Control	0	3.70	2.72	2.03	1.30	0.227	0.096	0.250
		5/5	5/5	5/5	5/5	2/2	2/5	2/5

\* Concentration was 50 mg./ml. (6.25 mg./Kg.), given intramuscularly in the rabbit.

† Control rabbits were given intramuscularly commercial oxytetracycline dry powder for reconstitution, 50 mg./ml. (6.25 mg./Kg.).

TABLE III

*Serum Levels,  $\mu\text{g./ml.}$ , Single Injection, in Human Beings of Oxytetracycline Intramuscular Solution, 100 mg.*

	Hours after dose		
	1	3	6
Average of 9 patients	0.88	1.14	0.63

of 1 ml. of solution containing 50 mg. of antibiotic. No residual drug was evident in the muscles, which exhibited slight irritation and induration when examined in the fresh state. After fixation some small lesions were found, but the condition of these muscles compared favorably with those of control animals that had received an equal number of injections of the dry powder formulation.<sup>2</sup>

#### CLINICAL STUDIES

Tolerance and blood level observations were made initially in a series of patients receiving oxytetracycline intramuscular solution without a local anesthetic. Twenty-eight persons were given 100 mg. of this preparation twice daily for 3 to 4 consecutive days. An additional 2 patients received four 100 mg. doses daily for three days, and another patient received three daily doses for 15 days. No evidence of local reaction was seen at any time. Moderate local pain accompanying and immediately following the injection was reported in approximately half the cases. Results of blood samples for antibiotic assay taken after the first intramuscular injection in 9 patients are shown in table III.

Subsequent clinical evaluations were conducted with the oxytetracycline solution containing lidocaine. In 10 persons a crossover was done with single 100 mg. injections of oxytetracycline as the solution and as the commercial dry-fill formulation. Tolerance, from the point of view of pain and tissue reaction, was equal and good for both preparations. A total of 48 persons were then studied for tolerance to multiple 100 mg. injections. This series consisted of 4 patients who received four doses daily for one to three days, 9 persons who received three doses daily for three days, and 35 persons who received two doses daily for three to seven days. Eleven of the persons on the twice a day schedule received the oxytetracycline solution in one buttock throughout the study and had simultaneous injections of the commercial dry-fill oxytetracycline in the contralateral buttock. Eighteen other patients receiving only commercial dry-fill oxytetracycline, 100 mg. two or three times daily for three days, at the time of these studies served as additional control subjects.

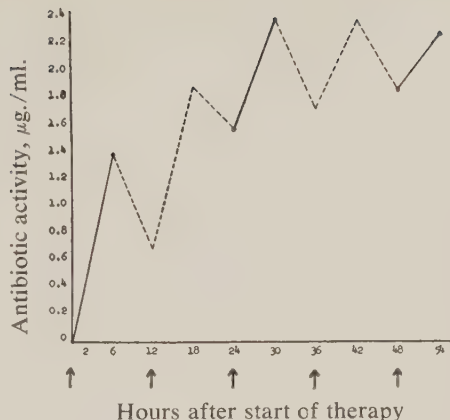
TABLE IV

*Serum Levels,  $\mu\text{g./ml.}$ , Multiple Injection in Human Beings of Oxytetracycline Intramuscular Solution, 100 mg. Every 12 Hours*

Hour of injection	0	12	24	36	48
Sample taken—hours after preceding dose	6	12*	6	12*	6
Sample taken—hours after first dose	6	24	30	48	54
Dry-fill, av. of 5 patients	1.1	1.5	1.9	1.3	2.2
Solution, av. of 5 patients	1.4	1.6	2.4	1.9	2.3

\* Twelve hour samples taken immediately before repeat dose.

FIG. 1. Serum levels with oxytetracycline solution, 100 mg. every 12 hours at times indicated by  $\uparrow$ , are given. — assayed levels; ---- probable levels.



Pain, either immediate or delayed, was not significant with either single or multiple doses. Where crossover or parallel studies were done with both oxytetracycline solution and dry-fill, both were equally well accepted by the patient. Some tenderness to palpation or mild induration were noted after four or more injections in 2 patients receiving the solution, in 5 receiving the dry-fill, and with both materials in 1. In no instance was serious local reaction observed.

Eighteen of the persons receiving the intramuscular solution had bacterial infections for which antibiotics were indicated. These included cases of cystitis, cellulitis, pneumonia, and upper respiratory infections. Good therapeutic responses were noted in these cases.

Blood samples taken from persons given multiple doses of oxytetracycline intramuscular solution or the dry-fill preparation are given in table IV. The continuous and cumulative nature of antibiotic levels with 100 mg. given twice daily is depicted in figure 1.

#### SUMMARY

After the successful completion of animal irritation and serum level determinations, a total of 89 persons were studied following the administration of single or multiple doses of a new, preconstituted oxytetracycline intramuscular solution. This formulation has been found to be well tolerated and to produce satisfactory antibiotic serum levels. Complete bio-potency retention for periods in excess of one year have been realized for this preparation.

#### BIBLIOGRAPHY

1. WRIGHT, W. A.; P'AN, S. Y., AND CARLOZZI, M.: Oxytetracycline intramuscular. *In: Antibiotics Annual 1953-1954*, New York, Medical Encyclopedia, Inc., 1954, pp. 137-140.
2. P'AN, S. Y.; KLIOZE, O.; GORDON, P. N.; JAROWSKI, C. I.; PILGRIM, F. J., AND HAMMER, H. F.: Oxytetracycline and tetracycline for intramuscular injection. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, pp. 634-636.
3. KATZ, M.; KLIOZE, O., AND P'AN, S. Y.: A study of parenteral oxytetracycline and tetracycline, *J. Am. Pharm. A. (Scient. ed.)* 44:751, Dec., 1955.

Although the broad-spectrum antibiotics have achieved a notable advance in the treatment of brucellosis, there still remains room for improvement if more desirable and optimal therapeutic results are to be obtained. The high incidence of recurrences in this disease may be attributed to the presence of intracellular *Brucella* organisms within the reticulo-endothelial system. This location renders the pathogens relatively inaccessible to soluble antibiotics, which are unable to trespass the cellular membrane. This assumption prompted Ruiz Castañeda<sup>5</sup> to have oxytetracycline\* prepared in the form of small insoluble particles to be administered by deep subcutaneous injection. These particles are assumed to be phagocytized by monocytes and polymorphonuclear leukocytes and carried to the spleen and lymph nodes, where the *Brucella* are harbored. The phagocytic carriers of the antibiotic may then be phagocytized, in turn, by the reticulo-endothelial cells. In this way, a higher antibiotic concentration becomes available at the site of active parasitism. A greater, more direct therapeutic effect may therefore be achieved, reducing the incidence of recurrences. Using this form of treatment, Ruiz Castañeda and Carrillo<sup>6,7</sup> treated 68 patients and Magriña et al<sup>3,4</sup> treated 35 patients. We followed the same method used by these investigators in the treatment of brucellosis, a disease that is very frequent in our area. The first report on our results was presented at the Clinical Session of the Faculty of Medicine of Zaragoza, January, 1957,<sup>1</sup> and a second report was presented at a meeting held in March, 1958.<sup>2</sup>

## MATERIAL AND METHODS

We utilized in our studies a specially prepared form of insoluble oxytetracycline containing 80 mg. of the antibiotic in each vial. Our treatment schedule consisted of the following: an initial injection of 80 mg. injected into the fatty tissue of the gluteal region (subcutaneously, deep), followed by simple weekly injections of 160 mg. for the duration of the treatment period.

After each injection mild pain is experienced at the site of injection and on the next day a minor febrile action occurs. On the average, we administered 8 to 10 injections, over an 8 to 10 week period of time, which represents a total dosage for the treatment period of 1.2 to 1.52 Gm. Because it is common in the course of brucellosis for patients to improve from time to time and even in certain cases to appear to be cured temporarily, it is exceedingly difficult to evaluate any particular therapy in the treatment of this condition. Therefore, we subjected our patients to the most careful type of control. The most exacting clinical and laboratory tests were carried out and continued for long periods after patients had achieved apparent clinical cure. We have included in our subject group reported here only those patients who were hospitalized, because all tests could thus be conducted with greater ease and reliability of results. In all patients we performed the following: (1) a blood culture examination to determine the presence of *Brucella* organisms (method of Ruiz Castañeda), performed every two weeks; (2) seroagglutina-

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\* The trade name of Chas. Pfizer & Co. for oxytetracycline is Terramycin.

TABLE I  
*Treatment of Brucellosis by Insoluble Oxytetracycline (58 cases)*

Case no.	Type of brucellosis	Duration of disease	Before treatment			Treatment		Response to therapy		
			Previous treatment*	Blood culture	Seroagglutination test	No. of injections	Total dosage, Gm.	Blood culture negative on day	Clinical improvement on day	Result
1	Acute	2 mo.	None	+	1/640	7	1.04	14	16	Cure
2	Acute	8 days	None	+	1/320	10	1.52	21	24	Cure
3	Acute	9 days	None	+	1/640	6	0.88	21	21	Cure
4	Acute	2 mo.	C	—	1/160	10	1.52	—	28	Cure
5	Chronic	3 mo.	C	—	1/160	7	1.04	—	21	Cure
6	Acute	3 mo.	T, C	+	1/320	10	1.52	28	31	Cure
7	Acute	1 mo.	None	+	1/160	12	1.84	21	28	Cure
8	Acute	19 days	None	+	1/160	8	1.20	28	30	Cure
9	Chronic	3 mo.	T	—	1/640	13	2.0	—	35	Cure
10	Acute	5 mo.	T, P, CT	+	1/160	6	0.88	No	No	Failure
11	Acute	24 days	None	+	1/160	8	1.20	21	21	Cure
12	Acute	3 mo.	None	+	1/320	9	1.36	21	28	Cure
13	Acute	25 days	None	+	1/1280	8	1.20	14	21	Cure
14	Acute	1 mo.	None	—	1/320	7	1.04	—	28	Cure
15	Acute	9 mo.	S, OT	+	1/160	9	1.36	21	30	Cure
16	Acute	1 mo.	CT	+	1/320	7	1.04	21	14	Cure
17	Acute	2 mo.	None	+	1/160	8	1.20	28	35	Cure
18	Acute	2 mo.	None	+	1/160	8	1.20	14	10	Cure
19	Acute	21 days	None	+	1/640	10	1.52	14	21	Cure
20	Acute	15 days	C	+	1/320	10	1.52	14	14	Cure
21	Acute	2 mo.	None	+	1/320	7	1.04	14	21	Cure
22	Acute	5 mo.	None	+	1/320	8	1.20	28	32	Cure
23	Chronic	2 yr.	P, S	—	1/160	8	1.20	—	28	Cure
24	Acute	2 mo.	None	+	1/1280	8	1.20	28	37	Cure
25	Acute	1 mo.	P	+	1/320	7	1.04	14	21	Cure
26	Chronic	2 yr.	P	+	1/1640	10	1.52	28	30	Cure
27	Acute	7 days	S	+	1/1280	8	1.20	14	21	Cure
28	Acute	3 mo.	None	+	1/1280	8	1.20	28	32	Cure
29	Acute	5 mo.	T, CT, C	+	1/640	7	1.04	28	28	Cure
30	Acute	3 mo.	None	+	1/640	10	1.52	14	21	Cure
31	Acute	2 mo.	None	—	1/640	8	1.20	—	14	Cure
32	Acute	5 mo.	Vaccine	+	1/1280	10	1.52	21	26	Cure
33	Acute	21 days	S	+	1/640	8	1.20	14	18	Cure
34	Acute	4 mo.	P, vaccine	+	1/640	10	1.52	21	26	Cure
35	Acute	4 mo.	P	+	1/160	5	0.72	21	28	Cure, recurrence
36	Acute	3 mo.	None	—	1/160	6	0.88	—	21	Cure, recurrence
37	Acute	2 mo.	None	+	1/320	6	0.88	14	18	Cure
38	Acute	10 days	None	+	1/320	7	1.04	21	25	Cure
39	Acute	20 days	None	+	1/640	10	1.52	14	18	Cure
40	Acute	1 mo.	S	+	1/160	5	0.72	14	20	Cure, recurrence
41	Acute	5 mo.	S, T, OT	+	1/160	7	1.04	21	26	Cure
42	Acute	5 mo.	S, C, OT	+	1/320	10	1.52	No	No	Failure
43	Acute	1 mo.	None	+	1/640	7	1.04	14	20	Cure, recurrence
44	Acute	6 mo.	Vaccine	+	1/640	10	1.52	14	26	Cure
45	Acute	3 mo.	P	+	1/320	7	1.04	21	25	Cure
46	Acute	1 mo.	None	+	1/160	8	1.20	14	18	Cure
47	Acute	3 mo.	None	+	1/160	8	1.20	14	17	Cure
48	Acute	8 mo.	OT	+	1/160	10	1.52	—	21	Cure
49	Chronic	9 mo.	CT	+	1/640	12	1.84	21	30	Cure
50	Acute	4 mo.	P, sulfon-amides	+	1/320	8	1.20	14	20	Cure
51	Acute	1 mo.	None	+	1/160	7	1.04	14	18	Cure
52	Acute	2 mo.	S	+	1/160	6	0.88	21	26	Cure, recurrence
53	Acute	3 mo.	None	+	1/320	8	1.20	14	22	Cure
54	Acute	1 yr.	CT, OT	+	1/320	8	1.20	21	29	Cure
55	Acute	20 days	None	+	1/160	8	1.20	14	18	Cure
56	Acute	40 days	None	+	1/320	9	1.36	14	20	Cure
57	Acute	2 mo.	None	+	1/640	10	1.52	21	31	Cure
58	Acute	3 mo.	T	—	1/1280	12	1.84	—	32	Cure

\* P = penicillin; S = streptomycin; T = tetracycline; C = chloramphenicol; CT = chlortetracycline; OT = oxytetracycline (oral).

TABLE II

*Laboratory Findings in 2 Patients Who Did Not Respond to Oxytetracycline Therapy*

Case no.	Seroagglutination test	Complement-fixation test	Incomplete antibodies
10	+1/160	+1/1280	+1/5120
42	+1/640 = 1/1280	+1/640	+1/10240

tion tests, performed every two weeks; (3) complement-fixation tests, performed every four weeks; (4) the Coombs' test for determining incomplete antibodies, performed every four weeks.

All serological tests were subjected to a double control in two different laboratories: first in our own laboratories of microbiology and parasitology in the medical school, and second, in the bacteriological department of the Municipal Hospital for Infectious Diseases of Barcelona. Patients were followed up as completely as possible. In many cases it was possible to recall patients to the hospital periodically for conducting the laboratory tests outlined. In those cases where it was impossible for the patient to return to the hospital, we maintained contact with the patient and by inquiry determined if his cure was complete and long-lasting.

## RESULTS

The total of 58 patients treated by us were admitted to two different hospitals: the Clinical Hospital of the Medical School and the Hospital Provincial, both of Zaragoza. The majority of our patients have had a continued follow-up after clinical cure of more than two years. In table I, we have reported the type and the duration of disease, previous therapy, dosage of oxytetracycline used, laboratory data, and clinical results. In 50 of our patients, we obtained a positive blood culture of *Brucella melitensis* and a diagnosis was based on this test. In the remaining 8 patients, the diagnosis of brucellosis was made on the clinical symptoms of the patient and laboratory tests performed as stated previously. In all but 2 patients (cases 10 and 42), cure was confirmed by the fact that the blood culture became negative uniformly during the second to fourth week of treatment. Other signs of improvement, such as disappearance of splenomegaly, return of temperature to normal, and improvement in well-being of the patient, occurred later but had a definite relationship to the disappearance of organism from the blood culture.

The two patients who did not respond to this form of oxytetracycline therapy had endocardial brucellosis with profound alterations in the electrocardiograph. One patient, case 10, had previously failed to respond to treatment with penicillin, tetracycline, and chlortetracycline. The other, case 42, had failed to respond to chlor-

TABLE III

*Second Course of Treatment with Insoluble Oxytetracycline after Recurrence (5 Cases)*

Case no.	No. of injections in previous treatment	Time elapsed between previous cure and recurrence, mo.	Blood culture (all negative during remission)	No. of injections for treating recurrence	Negative blood culture attained, days	Clinical improvement attained, days
35	5	6	+	7	14	16
36	6	4	—	7	—	10
40	5	2	—	8	—	8
43	7	3	+	6	14	17
52	6	11	+	7	21	28

amphenicol, streptomycin, and oxytetracycline given orally. Despite the administration of this new form of oxytetracycline to these 2 patients, the serological picture did not return to normal and remained as it is shown in table II. Neither of these patients came to us initially with a diagnosis of brucellosis; both of them were patients in the cardiac service and were sent to us incidentally for examination, at which time it was discovered that they had brucellosis. Despite the treatment administered to them, the endocarditis these patients had developed was sufficiently severe and their inability to respond to any therapy so great that an unsuccessful outcome resulted and both patients died.

Five patients who showed all evidence of being cured and were discharged from the hospital later developed another episode of brucellosis. All these patients responded excellently to a second course of therapy with insoluble oxytetracycline. The second course of therapy was followed by the same careful laboratory tests given during the initial course of therapy (see table III).

#### DISCUSSION

The small number of recurrences observed here demonstrates the effectiveness of this form of insoluble oxytetracycline in the treatment of brucellosis. When considered along with the 68 cases reported by Ruiz Castañeda and Carrillo and the 35 cases of Magriña and his associates, our study serves to corroborate the value of this therapeutic method. It is well known that brucellosis is characterized by repeated remissions and recurrences. Therefore, extensive controls were maintained to establish clinical improvement and cure. Of particular interest is the fact that all patients were carefully followed for prolonged periods of time after treatment. In agreement with these investigators, it is recommended that this method of treatment be used over prolonged periods of time. Perhaps its greatest value may be after a clinical and bacteriological cure is once obtained, since recurrences or reinfection may thus be prevented. In the 5 patients who experienced a recurrence of brucellosis after our treatment, 2 received only five injections, 2 received only six, and 1 received seven. This was the minimal dosage administered in the entire series. After a second course of treatment, final and lasting cure was achieved in all these 5 patients. Eight to 10 injections are considered to be the minimum that should be recommended for administration, and in the rest of the patients, at least this many injections were administered. As compared with other methods of treatment, this form of therapy affords ease of administration, lower dosage, economy, safety, and absence of any complicating side effects.

#### SUMMARY

1. Fifty-eight patients with brucellosis have been treated with low dosages of a specially prepared insoluble oxytetracycline. Treatment consists of an initial injection of 80 mg. followed by 160 mg. each week for a total of 8 to 10 weeks. Periodic clinical and laboratory controls (blood culture, serum agglutination, complement-fixation, Coombs' test) were performed. Our follow-ups were continued for two or three years after discontinuation of treatment.

2. In 56 patients (96.55 per cent), therapeutic results have been very satisfactory. In 5 of these cases recurrences occurred after treatment, but responded successfully to a second course of therapy.

3. Two cases (3.34 per cent), diagnosed as brucellosis endocarditis before treatment, were resistant to all antibiotic therapy.

4. Tolerance to this dosage form was excellent in all cases. No side effects were observed.

#### ACKNOWLEDGMENTS

I wish to express my gratitude to Dr. Foz Tena (Director of the Bacteriological Department, Hospital Municipal de Infecciosos, Barcelona), Dr. E. de la Figuera (Professor of Medicine, Medical School, University of Zaragoza), and Dr. Garcia Moya and Mr. Domingo Sanz (Laboratory of Microbiology, Medical School, University of Zaragoza), for their cooperation.

The oxytetracycline used in this study was kindly supplied by Chas. Pfizer & Co., Inc., New York, N. Y.

#### BIBLIOGRAPHY

1. GOMEZ LUS, R.: La Terramicina insoluble en el tratamiento de la brucelosis humana, Arch. Fac. Med. Zaragoza 2:335-336, 1957.
2. GOMEZ LUS, R.: Nuevas aportaciones al tratamiento de la brucelosis con Terramicina insoluble, Arch. Fac. Med. Zaragoza 3:549-550, 1958.
3. MAGRIÑA, N.; FOZ, A., AND MANZANARES, J. M.: Tratamiento de la brucelosis con el método de la Terramicina insoluble, según Ruiz Castañeda, Presented to IV Intern. Cong. Hyg. Med. Mediterraneas, Barcelona, September, 1953.
4. MAGRIÑA, N., AND FOZ, A.: Tratamiento de la brucelosis humana con el método de la Terramicina insoluble, Bol. d. Cons. Gral. Col. Med. Spain 84:39-45, 1954.
5. RUIZ CASTAÑEDA, M.: Evolución de métodos de tratamiento de la brucelosis, Gac. méd. de Mexico 2:135-142, 1952.
6. RUIZ CASTAÑEDA, M., AND CARRILLO, C.: A new approach to treatment of brucellosis, Am. J. M. Sc. 226:504-508, 1953.
7. RUIZ CASTAÑEDA, M.: Chemotherapy of brucellosis, World Health Organ. Bull. 16:443-446, 1957.

Since its introduction into clinical practice, oxytetracycline (Terramycin\*) has been a most valuable agent for the treatment of infections of the urinary tract, particularly because of its broad-spectrum action and its potency against those pathogens that commonly cause infections of this system. Generally, in conducting this study, our objective was to confirm the clinical value of oxytetracycline in acute and chronic nonobstructive urinary tract infections. Specifically, we were particularly interested in observing, under carefully controlled conditions, the effect of the drug against a type of infection ordinarily considered difficult to treat—chronic urinary tract infections in which localized foci of the infecting organism had been established and including a number of cases that had failed to respond to previous therapy with other antibiotics.

## MATERIALS AND METHODS

Our group consisted of 90 patients. All of them presented an acute or chronic infection of a section of the urinary tract. Oxytetracycline was used in the treatment of the following conditions: acute pyelonephritis, 6 cases; chronic pyelonephritis, 23 cases; acute cystitis, 19 cases; chronic cystitis, 25 cases; urinary infection after partial nephrectomy or pyelotomy because of calculus, 2 cases; and preparation for urological operations, 3 cases. In addition to oxytetracycline therapy, and as indicated in certain patients, other appropriate urological therapy was performed. This, of course, may make it more difficult to interpret the results. Finally, we should like to report that in many cases previous treatment with other antibiotics had been unsuccessful prior to the administration of oxytetracycline.

We have excluded from our study those patients who presented a urinary tract infection sustained by obstructive pathology: infected hydronephrosis related to the presence of a ureteral calculus, chronic cystitis sustained by neoplasm of the bladder. Acute or chronic infections of the prostate have not been considered. Within our experience, antibiotics are of no value in the treatment of such infections, and treatment remains primarily surgical.

Dosage of oxytetracycline was generally 1 Gm. daily. In a few cases 1.5 Gm. was given daily. Duration of treatment varied. The high efficiency of this rather moderate dosage of oxytetracycline probably results not only from the double effect that the drug has on kidney tissue, since it reaches the kidney through both the blood stream and the urine, but also because oxytetracycline is excreted in active form in the urine and the concentrations of the drug along the urinary pathways are very high. From our study, it was obvious that the accepted practice in certain circles of giving 2 Gm. a day and in certain cases perhaps more for the treatment of acute infections of the urinary tract is probably unnecessary. It appeared obvious that for the reasons just given, dosages in the range from 1 to 1.5 Gm. a day are fully effective and constitute adequate therapy for the treatment of acute infections of the urinary tract as well as chronic infections.

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\* The trade name of Chas. Pfizer & Co. for oxytetracycline is Terramycin.

Clinical response to therapy (disappearance of infectious symptomatology, subsidence of fever, clearing of the urine) served to determine the effectiveness of the drug therapy used. Once clinical recovery was achieved, patients were advised to rest for a short period of time before returning to work or resuming full normal activities.

An analysis of the results reveals that in acute urinary infections (25 cases), oxytetracycline brought about rapid recovery in all of our cases. However, it does not protect against reinfection (i.e., new outbreaks of cystitis or pyelonephritis). Such an occurrence developed in 6 of our patients. In each instance, the new infection was cured by a new course of treatment, sometimes combined with local therapy and sometimes not. In chronic infections, results were generally very favorable. In 34 of 48 patients, oxytetracycline made it possible for us to control stubborn infections satisfactorily. Almost half the patients in this category had previously received other antibiotics without satisfactory response. The relatively high percentage of favorable results in these chronic infections is impressive, since the problem of sterilizing foci in chronic infections is well known. Urinary tract infections that have existed for a long time are perhaps the most refractory because of the many foci of organisms that develop along the urinary pathways.

A case in which we used oxytetracycline preoperatively will be described here.

*Case A 7247.* A 65 year old patient suffered from prostatic hypertrophy for some time, which led to the development of a giant vesical diverticulum and a bilateral ureterohydronephrosis resulting from the compression of the lower part of the ureters. After micturition, the bladder still contained 1500 ml. of purulent urine. In principle, such a degree of urinary tract infection would contraindicate a one-step operation (prostatectomy plus diverticulectomy). The patient received 1.5 Gm. of oxytetracycline daily, and at the same time a retaining catheter was introduced. Four days after this treatment was begun, the urine was practically clear and the sediment contained only 10 white cells/field. A one-step operation was then performed and the patient was permitted to leave the clinic 20 days later.

This case illustrates the marked clinical value of oxytetracycline.

The problem of nongonococcal urethritis is one of the most difficult to be encountered in urological practice. As is known, it includes *Trichomonas* urethritis, urethritis with innocuous germs, virus urethritis, urethritis with "L" organisms, and last, chemical urethritis. A distinction between these various types can be made only with the help of a highly specialized bacteriological laboratory. Here also, we should like to state that antibiograms made with cultures taken from the urethra were of little practical value. In our experience, these examinations were not confirmed clinically. Most of the commonly used antibiotics have received clinical trial in this condition by us. The results obtained were frequently disappointing. Our results in nongonococcal urethritis with oxytetracycline lead us to believe that the patients should be treated at the outset with massive, prolonged dosage, whenever possible.

Antibiograms seem to lack practical value. Very often, we have noted a manifest contradiction between the data provided by the antibiogram and the results obtained on a clinical level. Of course, there is no doubt that the identification of the pathogenic agent is of great interest, but decisions as to the course and type of treatment to be used should not be based on this consideration alone.

Side effects with oxytetracycline were infrequent (9 cases) and benign. The complaints, which were minor, were essentially of a digestive nature (glossitis or diarrhea). They were characterized by immediate reversibility as soon as the treatment was stopped.

We were not able to determine whether preventive vitamin therapy or vitamin therapy combined with the treatment has any effect on the incidence or degree of side effects.

#### SUMMARY

Oxytetracycline was employed in 90 patients for treatment of acute or chronic urinary tract infections. Patients with obstructive pathology were not selected for study.

The usual dosage of oxytetracycline was 1 Gm. daily. Results were generally favorable, and we were particularly impressed with the high rate of success we observed in the treatment of chronic urinary tract infections where foci of pathogens had been established and previous antibiotic therapy with other agents had failed. In contrast with the findings of authors who have previously studied the same problem, we find the antibiogram of questionable value.

We encountered side reactions, which were primarily gastrointestinal in nature. Such reactions are not associated solely with the use of oxytetracycline, but can occur with other antibiotics as well.

# Extended Low-Level Dosage of Oxytetracycline

## II. Progress Report

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In the first part of this study, reported in October, 1957, it was demonstrated that 50 mg. of oxytetracycline could be ingested daily during a period of at least one year without provoking adverse effects, including specific antibiotic sensitization, or the emergence of resistant strains of *Staphylococcus aureus* and enterococci. The second part of this study, currently in progress and as yet incomplete, was designed to determine possible quantitative reductions in the sensitivity of intestinal aerobes, *Staph. aureus* and coliforms to a range of concentrations of oxytetracycline that might be caused by further extended low-level dosage of this antibiotic.

### TEST MATERIAL AND METHODS

One hundred twenty school children—half of whom had been taking 50 mg. doses of oxytetracycline daily during a period of 21 months and half of whom had received no antibiotic previously—were selected for this study.

After making initial quantitative determinations of the sensitivity of the aforementioned bacterial flora to 0, 5, 10, 15, and 20  $\mu\text{g.}/\text{ml.}$  concentrations of oxytetracycline (*vide infra*), the children were separated further into four groups. The 50 mg./day dosage of oxytetracycline then was discontinued for 30 of those who had been receiving these doses of the antibiotic for 21 months. The dosage of 50 mg./day of oxytetracycline was continued for the other 30 children who had been receiving this antibiotic for 21 months. Oxytetracycline, in daily 50 mg. doses, was started in a third group of 30 children who had received no antibiotic previously. For the last group of 30 children, who also had received no oxytetracycline previously, no antibiotic was given, and thus they were considered as controls.

Micro-suspensions of feces of all of the children, obtained by serially diluting the material with physiological salt solution, were added to poured plates of individual selective media, each made up to result in 0, 5, 10, 15, and 20  $\mu\text{g.}/\text{ml.}$  of oxytetracycline activity. After incubation for prescribed periods depending on the media and bacteria being cultured, counts of bacterial colonies were made of each poured plate.

As just stated, colony counts were made initially before the children were separated into the four study groups and then were repeated at one month and three months later. Other similar quantitative determinations for sensitivity of these fecal flora to oxytetracycline were planned for the fifth and seventh months of the study.

### RESULTS

According to a preliminary analysis of the data that has been accumulated thus far (children in the second group received 50 mg. daily doses of oxytetracycline for 24 months)—tabulations of the data of each group will be made at the com-

pletion of the study—the following have been noted: (1) there have been no definitive shifts of sensitivity of fecal aerobes to these test concentrations of oxytetracycline; (2) there has been no material change in the frequency of *Staph. aureus*; (3) there has been no consistent reduction of sensitivity of *Staph. aureus* to these test concentrations of oxytetracycline; (4) there has been no material change in the sensitivity of the coliforms to these test concentrations of oxytetracycline; and (5) none of the children, including the 30 who have received 50 mg./day dosages of oxytetracycline for 24 months, have had any adverse reactions toward this antibiotic.

#### BIBLIOGRAPHY

1. LOUGHLIN, E. H.; ALCINDOR, L., AND JOSEPH, A. A.: Extended low-level dosage of oxytetracycline. *In*: Antibiotics Annual 1957–1958, New York, Medical Encyclopedia, Inc., 1958, p. 95.

# Observations on the Distribution of C<sup>14</sup> Oxytetracycline in Man

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Considerable information has been accumulated on the metabolism of antibiotics in man, using microbiological techniques. Radioactive penicillin,<sup>1</sup> streptomycin,<sup>2</sup> and oxytetracycline<sup>3</sup> have greatly facilitated studies of absorption, distribution, and excretion of these agents in both experimental animals and man. Further investigations using these tagged antibiotics are needed in human beings to determine if results of animal studies are applicable to man. This paper compares antibiotic blood and urine levels obtained by radiochemical and microbiological analyses after receipt of random labeled C<sup>14</sup> oxytetracycline and records observations on its distribution and excretion in healthy subjects and selected patients with liver and kidney disease.

## MATERIALS AND METHODS

Tagged oxytetracycline, 250 mg., prepared by the method of Snell et al<sup>4</sup> (specific activity about 0.1  $\mu$ C./mg.), was given orally to 17 subjects, 8 of whom had no evidence of disease, 6 with cirrhosis, and 3 with renal dysfunction. Antibiotic levels were determined in collected aliquots of blood, urine, bile, and feces by both liquid scintillation radiochemical analyses and microbiological techniques. Hepatic metabolism of the antibiotic was studied using the technique of hepatic vein catheterization<sup>5</sup> and by obtaining percutaneous liver biopsies 1, 6, and 24 hours after its administration.<sup>6</sup>

## OBSERVATIONS

There was no correlation between results of radiochemical and microbiological analyses on the same samples of peripheral venous blood, arterial blood, hepatic venous blood, urine, or bile after oral administration of a single capsule of 250 mg. of tagged oxytetracycline (table I). Patterns were similar during the first 12 hours, with a rapid increase in both levels to reach a peak in two to four hours. Higher antibiotic levels by radioisotopic techniques were attributed to greater sensitivity of this method; the fact that a significant portion of the antibiotic is bound to proteins and cellular elements of the blood and may not be detected by microbiological studies;<sup>3</sup> and the presence of nonactive breakdown products of oxytetracycline, which contribute to measured radioactivity. Fluctuation in antibiotic levels in the first 12 hours occurred with both methods due to variation in absorption, release from tissue stores, and biliary and urinary excretion. After 12 hours, although the antibiotic could not be detected in the serum by microbiological studies, radiochemical analysis showed considerable amounts, probably initially reflecting the presence of bound drug, since both methods demonstrated the antibiotic in urine up to 96 hours. Subsequently, when urine no longer exhibited

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TABLE I

Comparison of Radiochemical (Expressed in d/m/ $\mu$ g.) and Microbiological Analyses (Expressed in  $\mu$ g./ml.) after  $C^{14}$  Oxytetracycline (194 d/m/ $\mu$ g.)

	Case 1					Case 2				
	Plasma		Urine			Plasma		Urine		
	d/m/ $\mu$ g.	$\mu$ g./ml.	d/m/ $\mu$ g.	$\mu$ g./ml.	Quant., ml.	d/m/ $\mu$ g.	$\mu$ g./ml.	d/m/ $\mu$ g.	$\mu$ g./ml.	Quant., ml.
Fasting	6.5	0	0.0	0	175	0.0	0	0.0	0	85.0
1 hour	155.5	0.480	6,387.0	15.9	130	149.2	0.168	470.3	14.7	58.0
2 hours	152.4	0.615	265,000.0	30.4	100	88.6	0.519	1,754.0	31.0	30.0
3 hours	471.5	0.555	65,651.0	30.7	30	223.8	0.340	—	—	—
4 hours	117.9	0.519	40,412	30.8	75	267.7	0.411	91,785.2	30.8	50.0
8 hours	388.5	0.294	—	—	—	96.0	0.294	—	—	—
12 hours	82.0	0	—	—	—	48.9	0	—	—	—
24 hours	69.1	0	14,111	27.0	1,780	77.4	0	5,596.3	15.3	1,263
48 hours	381.7	0	3,665.2	4.1	1,500	151.7	0	5,851.4	6.6	1,210
3 days	175.9	0	1,112.6	1.77	610	556.4	0	531.2	1.23	1,500
4 days	134.3	0	1,138.0	0.267	1,360	0.0	0	227.5	0.135	1,800
5 days	321.2	0	590.0	0	1,320	51.3	0	110.6	0	1,300
6 days	19.6	0	212.0	0	990	95.5	0	527.7	0	1,300
7 days	37.0	0	533.0	0	960	210.6	0	203.3	0	1,110

detectable inhibiting effects on test microorganisms, both plasma and urinary radioactivity were largely due to metabolites with little or no biological activity.

Under conditions of hepatic vein catheterization, initial peripheral blood levels of  $C^{14}$  oxytetracycline in healthy subjects resulted from lymphatic absorption as detection of oxytetracycline in hepatic venous blood is delayed (fig. 1). This finding has been confirmed in normal subjects, using nonradioactive oxytetracycline.<sup>7</sup> Comparison of antibiotic levels in venous, arterial, and hepatic venous blood shows a small but definite difference due to its uptake and storage by the liver and other tissues, and excretion by the kidney. Radioactivity appeared in the bile within one hour and usually reached a maximum level in 2 to 3 hours; likewise, urinary radioactivity was prominent in one hour and maximum in 3 to 8 hours. Hepatic storage of the labeled antibiotic was also demonstrated by serial liver biopsies obtained in 3 subjects without clinical, functional, or histological evidence of liver disease. Tissue radioactivity was much higher than that in the plasma (table II).

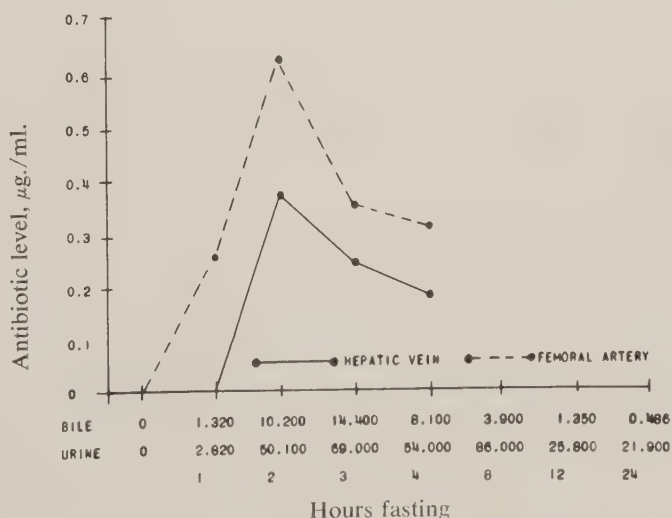


FIG. 1. Antibiotic levels obtained with  $C^{14}$  oxytetracycline in normal subject under conditions of hepatic vein catheterization.

TABLE II

*Radioactivity in Percutaneous Liver Biopsies after Administration of C<sup>14</sup> Oxytetracycline (194 d/m/μg.) in Healthy Subjects*

Patient	Time, hr.	Peripheral blood, μg./ml.	Liver biopsy, μg./ml.
J. R.	1	0.21	88.0
	6	1.24	62.70
A. W.*	1	0.63	0.00
	6	1.28	38.2
	24	0.39	33.7
L. Q.†	1	0	116.6
	6	3.76	18.9
	24	1.03	192.0

\* After previous non-labeled oxytetracycline. The absence of radioactivity in the first biopsy may be due to saturation of the hepatic depot with nonradioactive oxytetracycline.

† The decrease in radioactivity in the 6 hour specimen may be related to sampling differences.

In patients with cirrhosis and vascular shunts, there appeared to be a diminished uptake of C<sup>14</sup> oxytetracycline by the liver, as reflected in its earlier appearance in the hepatic venous blood and a smaller hepatic vein-arterial difference (fig. 2). Similar results were noted using microbiological assays in patients with cirrhosis receiving nonradioactive oxytetracycline. This phenomenon tended to increase temporarily the plasma level of the antibiotic; biliary excretion was maintained, and this, coupled with urinary losses, prevented marked rises in plasma level. A patient with chronic glomerulonephritis and 1 with prerenal and postrenal azotemia had a marked diminution in anticipated urinary losses of C<sup>14</sup> oxytetracycline. Unlike the patient with complete anuria, in whom there may be persistent elevation of antibiotic blood level due to failure of the renal excretory mechanism,<sup>8</sup> plasma levels in these patients were similar to those seen in cirrhosis, presumably due to its extracellular distribution and tissue storage (table III).

#### SUMMARY AND CONCLUSIONS

1. Comparative radiochemical and microbiological analyses of blood and urine antibiotic levels after administration of C<sup>14</sup> oxytetracycline show marked differences

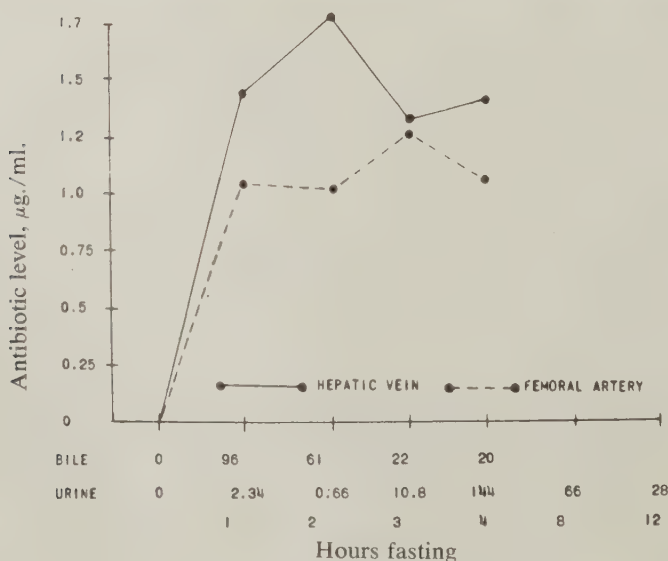


FIG. 2. Antibiotic levels obtained with C<sup>14</sup> oxytetracycline in patient with moderately advanced cirrhosis under conditions of hepatic vein catheterization.

TABLE III

*Urinary Excretion of C<sup>14</sup> Oxytetracycline in Renal Dysfunction (193 d/m/μg.)*

	Case 1*			Case 2†		
	Plasma	Urine		Plasma	Urine	
	d/m/μg.	d/m/μg.	Volume, ml.	d/m/μg.	d/m/μg.	Volume, ml.
Fasting	0	0		0	0	
1 hour	131.5			244.9	0	40
2 hours	1,628			205.9	30.4	90
3 hours	174.4	220	170	112.2	420.0	45
7 hours	58.5	230.9	200	108.2	1,151.8	60
12 hours	30.0			838		
24 hours	13.6	116.2	1,100	159.1	1,226.3	1,200
48 hours	6.5	7,043.4	1,450	219.0	530.4	1,200
72 hours	16.0	2,909.5	1,250	57.4		
96 hours	30.1	2,097.0	1,350	147.0	301.6	2,550

\* C<sup>14</sup> oxytetracycline and plasma levels and urinary excretion in patient with nephrotic syndrome and azotemia due to chronic glomerulonephritis.

† C<sup>14</sup> oxytetracycline plasma levels and urinary excretion in patient with massive gastrointestinal hemorrhage and azotemia.

in healthy men. This is attributable to greater sensitivity of the radioisotopic method, inability of the microbiological technique to detect antibiotic bound to protein or cells, and measurement of inactive breakdown products by radiochemical analyses.

2. Peripheral blood levels of oxytetracycline appear to depend upon its rate and degree of absorption, tissue storage, and urinary excretion. Hepatic storage can be demonstrated in normal subjects by arterial-hepatic vein drug differences and measurement of radioactivity in liver biopsy specimens after administration of the tagged antibiotic.

3. In patients with hepatic cirrhosis and vascular shunts there was diminished hepatic uptake of the drug, but this was not of the magnitude to alter significantly either serum levels, biliary excretion, or urinary losses. In contrast, patients with renal dysfunction without anuria had a marked decrease in amounts of the antibiotic recovered in the urine.

#### ACKNOWLEDGMENT

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#### BIBLIOGRAPHY

1. ROWLANDS, S.; ROWLEY, D., AND STEWART, H. C.: Absorption and excretion studies with radioactive penicillin, *Lancet* 2:491, 1948.
2. ANDRE, T.: Studies on the distribution of tritium-labeled dihydrostreptomycin and tetracycline in the body, *Acta radiol. Supp.* 142, 1956.
3. SNELL, J. F.; GARKOSCHA, R., AND ALLEN, E. L.: Radioactive oxytetracycline. II. Distribution in C-57 BL mice (preliminary experiments). In: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 502.
4. SNELL, J. F.; WAGNER, R. L., JR., AND HOCKSTEIN, F. A.: Radioactive tetracycline. I. Mode of synthesis and properties of the radioactive compound, *International Conference on Peaceful Uses of Atomic Energy*, Aug., 1955, p. 131.
5. LEEVY, C. M., AND GLIEDMAN, M.: Practical and research value of hepatic vein catheterization, *New England J. Med.*, 258:696-738, 1958.
6. LEEVY, C. M.: *Practical Diagnosis and Treatment of Liver Disease*, New York, Paul B. Hoeber, Inc., 1957.
7. LEEVY, C. M.; ZINKE, M.; CHEY, W. Y.; SNELL, J., AND ENGLISH, A.: Observations on hepatic metabolism of oxytetracycline in man. *Abstr. J. Clin. Invest.* 37:910, 1958.
8. KUNIN, C., AND REES, S. R.: Persistence of antibiotics in blood of patients with acute tubular necrosis and uremia, *J. Clin. Invest.* 37:908, 1958.

# Topical Use of Antibiotics in the Treatment of Chronic Skin Ulcers

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It has long been known that the self-repairing process after losses of skin substances is influenced by different factors that help or inhibit cicatrization of the tissues. These factors have been exhaustively studied by many investigators, as seen from the large bibliography on this subject.

In this paper we shall refer exclusively to the local use of antibiotics in the treatment of chronic ulcers of the skin and we shall show the results obtained.

The infectious process, even when well localized, must be considered not only as a potential focus, as a starting point of possible diffused or generalized infection, but also as a contributing factor in inhibition of the self-repairing capacity of the tissues.

At the beginning of this study, we thought that by using antibiotics in selective form, we could avoid generalized infections and that we could maintain the tissues in the best condition for repair after loss of substances, spontaneously in the case of small lesions and as a form of protection in the case of larger lesions requiring plastic repair.

## MATERIALS AND METHODS

This investigation is based on observation of 75 patients with symmetrical bilateral lesions. They were studied from June, 1957, to May, 1958. There were 50 male and 25 female patients. The ages of the patients were: 0 to 5 years, 4 patients; 5 to 15 years, 14 patients; 15 to 30 years, 35 patients; 30 to 45 years, 15 patients; older than 45 years, 7 patients.

The causes of the chronic ulcerations of the skin were: burns, 35 cases; varicose veins, 14 cases; arterial circulatory disturbances, 5 cases; lesions produced by the administration of norepinephrine, 15 cases; surgical wounds, 6 cases.

The patients were divided into three groups, according to time of development of the ulcer: group 1, between 50 and 70 days, 10 cases; group 2, between 70 and 160 days, 50 cases; group 3, between 160 and 545 days, 15 cases.

The presence of local infection was clinically suspected and diagnosed in every case by bacteriological examination. Bacteriological cultures were done, using the infectious exudation that covered the granulations and also the same granulation tissue and the edges of the lesion. Bacteria found were: hemolytic *Staphylococcus aureus*, hemolytic *Streptococcus*, *Proteus vulgaris*, nonhemolytic *Streptococcus*, nonhemolytic *Staph. aureus*, *Alcaligenes faecalis*, *Proteus mirabilis*, *Pseudomonas fluorescens*, hemolytic *Staphylococcus albus*, nonhemolytic *Staph. albus*, *Escherichia coli*.

In 60 per cent of the cases, the bacteria found in the lesions were the same as those found in the sebaceous and sudoriferous glands of the skin surrounding the lesion and the more removed region of the zone of loss of tissue. The organisms were cultured in tryptose phosphate broth, containing tryptose 20 Gm., dextrose 2 Gm., sodium chloride 5 Gm., disodic phosphate 2.5 Gm.

With the object of demonstrating the susceptibility of the organisms to the anti-

biotics used, we made a diffusion antibiogram in all cases. The susceptibilities were: to triple sulfonamides, 0 per cent; to penicillin, 5 per cent; to dihydrostreptomycin, 6 per cent; to oleandomycin, 45 per cent; to novobiocin, 55 per cent; to tetracycline, 69 per cent; to erythromycin, 75 per cent; to oxytetracycline, 93 per cent; to chloramphenicol, 94 per cent. We considered only sensitivity, not taking inhibition into account. We believe that by using the therapeutic agent in the same medium in which the studies were made, the results could be better evaluated.

We prepared seven solutions of antibiotics all in 1 liter of bidistilled water: 3,000,000 units penicillin, 2 Gm. dihydrostreptomycin, 2 Gm. erythromycin, 2 Gm. novobiocin, 2 Gm. tetracycline, 2 Gm. oxytetracycline, 2 Gm. chloramphenicol.

After superficial cleansing with bidistilled water, we placed in the lesions on the right side dressings soaked in oxytetracycline solution, and in the lesions on the left side we applied the other solutions indiscriminately. We did not use antibiotic therapy in 6 patients and instead we used the best-known techniques for this type of lesion (wet dressings, petrolatum, boric acid). The dressings were replaced every eight hours, without any mechanical cleansing of the lesions. Bacteriological control cultures were repeated every 24 hours in order to check the action of the therapeutic agents used.

## RESULTS

The patients treated without antibiotic solutions showed positive cultures at all observations, although the amount of bacteria was slightly less than at the first analysis. The exudate edema, pain, and pruritus disappeared and there were small, rose-colored granulations in all patients treated with oxytetracycline, chloramphenicol, and erythromycin.

Sterile cultures were observed in 93 per cent of the patients treated with oxytetracycline, 72 per cent of the patients treated with chloramphenicol, 33 per cent of the patients treated with erythromycin, 10 per cent of the patients treated with tetracycline. In our opinion, the most important finding of this study is that in an average of only 56 hours we obtained sterile lesions with oxytetracycline, 85 hours with chloramphenicol, 114 hours with tetracycline, and 160 hours with erythromycin.

Of the 6 patients treated without antibiotics, the lesions were smaller than 30 cm. in 2 cases, and there was no spontaneous epithelization in these cases. The lesions were larger than 30 cm. in 4 cases. The 6 cases received autografts with an average take of 60 per cent of the area.

Sixty-nine patients were treated with antibiotics. The lesions were smaller than 30 cm. in 10 cases, and there was spontaneous epithelization in all 10. The lesions were larger than 30 cm. in 59 cases, all of which received split-skin autografts. In these cases, the graft take averaged 98 per cent of the area.

## SUMMARY

In 75 cases, the topical application of various antibiotics was evaluated in the management of infected chronic ulcerations of the skin, resulting from burns (35 cases); varicose veins (14); arterial circulatory disturbances (5); lesions produced by the administration of norepinephrine (15); and surgical wounds (6). The average duration of lesions prior to therapy was four months. A total of 69 patients were treated, some with multiple lesions. Bacteriological studies and antibiotic sensitivity tests were performed in all cases. Microorganisms were demonstrated that are

common to chronic skin ulcers (*Staphylococcus*, *Streptococcus*, *Pseudomonas*, *E. coli*, and *Proteus*). According to sensitivity tests, 93 per cent of the organisms were susceptible to oxytetracycline and chloramphenicol; 75 per cent to erythromycin; 69 per cent to tetracycline; and 55 per cent to novobiocin. Treatment consisted of application of dressings soaked in solutions of the various antibiotics being tested. Dressings were replaced every eight hours. Bacteriological control cultures were repeated every 24 hours.

Ulcers became sterile in the following percentage of cases and in the following average times: 93 per cent of ulcers treated with oxytetracycline, in 56 hours; 72 per cent with chloramphenicol, in 85 hours; 10 per cent with tetracycline, in 114 hours; and 33 per cent with erythromycin, in 160 hours.

Epithelization and complete healing were achieved in 30 per cent of the treated cases. The remainder required skin grafts, which were uniformly successful. Oxytetracycline proved to be the most efficient of the seven therapeutic agents tested; sterilization time was considerably shorter than with the other solutions.

#### BIBLIOGRAPHY

1. ALDRICH, F. H.: The role of infections in burns. The theory and treatment with special reference to Gentian Violet, *New England J. Med.* 208:299, 1933.
2. HUMMEL, R.; RIVERA, J., AND ARTZ, C.: Evaluacion de algunos antibióticos usados localmente en heridas granulates causadas por lesiones térmicas, *An. de cir.* p. 752, Nov., 1957
3. BULL, J. P., AND JACKSON, D. M.: The recent burn, *Lancet* 266:161, 1954.
4. LIEBERG; REIS; KUHN; AMSPACHER, AND ARTZ: Infection in burns. Evaluation of the local use of chloramphenicol ointment and Furacin (r) soluble dressing on granulation surface following extensive full thickness burns, *Surg., Gynec. & Obst.* 100:219, 1955.
5. MATTHEWS, D. N.: Burns, *Ann. Roy. Coll. Surgeons* 10:114, 1952.

# Triacetyloleandomycin: Its Use in the Treatment of Acne and Pyodermas Caused by Resistant *Staphylococcus aureus*

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The treatment of acne has been far less than satisfactory. However, antibiotics, although not curative, have an established place in the management of the pustular stages of acne.<sup>1-8</sup> To our knowledge, the use of triacetyloleandomycin\* to control pustular acne has not been reported.

Since the incidence of staphylococcal infections resistant to antibiotic therapy has been increasing at an alarming rate, further observations with new antibiotics seemed worth while. This is a preliminary report of our experience with triacetyloleandomycin in the management of pustular acne and the treatment of pyodermas caused by coagulase-positive *Staphylococcus aureus*, resistant to one or more antibiotics.

## SELECTION OF PATIENTS

Acne patients were placed on triacetyloleandomycin therapy if they had pustular lesions that did not respond to routine diet, acne surgery, ultraviolet light, and local sulfur resorcinol preparations. Triacetyloleandomycin was then added to the previous regimen. There was only one exception to this rule: A young girl with pustular acne and keratitis was given triacetyloleandomycin without the other therapy while the keratitis was being treated by ophthalmologists. The pustular acne improved considerably.

The cases of pyoderma selected were caused by coagulase-positive *Staph. aureus* resistant to one or more antibiotics. In one instance, the patient was sensitive to penicillin but his organism was not.

## RESULTS

The results are presented in table I. All patients were benefited to some degree, most of them considerably. All patients with pustular acne were treated with 250 mg. of triacetyloleandomycin twice daily for one to two weeks and then maintained on 250 or 125 mg. daily thereafter. When therapy was discontinued, the lesions relapsed within a week. Resumption of therapy invariably brought about improvement again.

Results were considered excellent if the pustular element was completely controlled, good if this factor was markedly reduced, and fair if there was improvement but continuation of pustules.

The infections with resistant *Staph. aureus* were treated with 250 mg. of triacetyloleandomycin four times daily until the infection was under control. This varied from five to seven days for furunculosis up to three weeks for a resistant pustular eruption of the feet. The latter patient still had some pustules after three weeks but they were completely sterile.

There were no adverse reactions to the triacetyloleandomycin, and in no instance was it necessary to discontinue it or to resort to another antibiotic to achieve favorable results.

\* The trade name of Wyeth Laboratories for triacetyloleandomycin is Cyclamycin.

TABLE I  
Results of Triacetyloleandomycin Therapy

Diagnosis*	No. cases	Results			Remarks
		Excellent	Good	Fair	
Pustular acne	40	25	9	6	One patient had pustular acne and keratitis; both improved on triacetyloleandomycin therapy alone
Recurrent furunculosis	10	10			One patient had poikiloderma atrophicans vasculare and another had diabetes
Persistent recurrent pustular eruption of the hands	4	4			One of these patients was sensitive to penicillin but his organism was not; one of these had recurrent pustular lesions of the hands for one year
Persistent pustular eruption of the hands and feet	2	2			Infection controlled on therapy, with relapse when it was stopped; controlled again with triacetyloleandomycin
Persistent pustular eruption of feet	4	4			One had diabetes; one had this condition for three years; one had lymphangitis and inguinal lymphadenopathy also
Infections of the nar space of the hand	1	1			This patient also had pustular acne; both conditions responded well to triacetyloleandomycin
Total	61	46	9	6	

\* Except for the acne, the organism in each instance was hemolytic, coagulase-positive *Staph. aureus*. All except 1 was resistant to penicillin, 9 were resistant to chlortetracycline and tetracycline, 9 were resistant to oxytetracycline, and 3 were resistant to erythromycin.

#### DISCUSSION

Repeated cultures from lesions of acne rarely reveal known pathogens, yet antibiotics seem to influence favorably the majority of pustular acne lesions while therapy is being given. A possible explanation of this would seem to be that organisms normally present on the skin, such as acne bacillus and *Staphylococcus albus*, may play a role in pustular acne and that these organisms are suppressed by the antibiotic therapy. This would seem to explain the beneficial effect during antibiotic therapy and the rapid relapse when it is discontinued.

Preliminary studies by us suggest strongly that the acne bacillus and *Staph. albus* may be important factors in pustular acne. Anaerobic cultures from some acne lesions have revealed the presence of one or both of these organisms fairly consistently. On the other hand, these organisms could not be demonstrated while the patient was receiving triacetyloleandomycin. The acne bacillus and *Staph. albus* reappeared when triacetyloleandomycin was discontinued and when the pustular lesions recurred.

It is interesting that Fleming<sup>9</sup> in 1909 also felt that the acne bacillus and *Staph. albus* might play an important role in acne. We were not aware of Fleming's paper, however, until this one was already in preparation.

#### CONCLUSIONS

1. Triacetyloleandomycin is very effective in controlling the pustular lesions of acne.
2. Triacetyloleandomycin is effective in the management of infections caused by *Staph. aureus* that is resistant to some other antibiotics.

3. Triacetyloleandomycin, in this series, was remarkably free of adverse reactions.

4. An explanation of the effectiveness of antibiotics in controlling pustular acne is offered.

#### ACKNOWLEDGMENT

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#### BIBLIOGRAPHY

1. REIN, C. R.; MANN, C. H.; OSEROFF, B. J.; BODIAN, E. L.; BUCKWALTER, F. H.; FLAX, S. L., AND NOEL, R. H.: A clinical evaluation of tetracycline in pustular dermatoses. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 563.
2. KING, W. C., AND FORBES, M. A., JR.: Clinical trial of orally administered tetracycline in the management of acne vulgaris: preliminary report. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 570.
3. APPEL, B.: Oxytetracycline and polymyxin B: their combined effect on pyodermatoses. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 949.
4. ROBINSON, H. M., JR.: Role of antibiotics in therapy of acne, *A.M.A. Arch. Dermat. & Syph.* 69:414, 1954.
5. VAN DE ERVE, J., JR.: Erythromycin in therapy of acne vulgaris, *J. Invest. Dermat.* 23:67, 1954.
6. BACKUS, H. H.: The treatment of acne vulgaris, *New York J. Med.* 50:1917, 1950.
7. BERNARD, R. D., AND ORENS, L. R.: Terramycin therapy in acne vulgaris, *New York J. Med.* 52:1528, 1954.
8. ANDREWS, G. C.; DOMONKOS, A. M., AND POST, C. F.: Treatment of acne vulgaris, *J.A.M.A.* 146:1107, 1952.
9. FLEMING, A.: On the etiology of acne vulgaris and its treatment by vaccine, *Lancet* 1:1035, 1909.

# Antibiotic Therapy of Acute Gonococcal Infections

## A Study of the Comparative Efficacy of Triacetyloleandomycin, Erythromycin, and Penicillin V in the Treatment of Acute Gonococcal Urethritis

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In the treatment of acute gonorrhea in men, most of the antibiotics currently in use are highly effective, provided optimal dosages are used. This is substantially true, whether limited or broad-spectrum antibiotics are given. Although gonococci have been exposed to antibiotics for many years, serious emergence of antibiotic-resistant strains has not occurred. The selection of the particular antibiotic to be given, therefore, would be determined by its cost, ease of administration, the incidence of adverse reactions provoked by it, and the probable cooperation of the patient. Ideally, the antibiotic should be curative when administered in a single dose, irrespective of the route of administration.

In this paper, we report the results of treatment of acute gonococcal urethritis with three antibiotics having similar therapeutic antimicrobial spectrums—triacetyloleandomycin, erythromycin, and phenoxymethyl penicillin—each administered by the oral route.

### DOSAGE

In view of the fact that certain broad-spectrum antibiotics are highly effective in the treatment of acute gonorrhea, when administered orally in 1 Gm. doses, we decided to find out if the same dose of triacetyloleandomycin would be just as effective. Thus, after determining that such was the case, we also set the dosage of erythromycin (given as the stearate) at a single dose of 1 Gm. However, considering our previous experience with the oral dosage of penicillin, as well as that of others, we felt that this antibiotic should be given in multiple doses for maximal effectiveness. Accordingly, the oral dosage of phenoxymethyl penicillin was set at 600,000 units to be given three times at six hour intervals for a total dose of 1.8 million units.

### DIAGNOSTIC AND THERAPEUTIC CRITERIA

The diagnosis of acute gonococcal urethritis was determined by a history of exposure, local physical examination, and finding gonococci in Gram-stained spreads of urethral exudate. Only men with the acute stage of gonorrhea were selected for evaluating these antibiotics.

The patients, after receiving their dosage of the antibiotics, were told to return for re-examination on the second or third days post-treatment and also on the seventh and fourteenth days post-treatment. At these times, spreads and cultures of urethral exudate or discharge, if any, were made and examined for gonococci. When bacteriological examinations were negative at the first two post-treatment

visits and the clinical manifestations of infection had disappeared, cure was thought to have been accomplished.

#### THERAPEUTIC RESULTS

Using the previous criteria for determining the results of treatment of acute gonorrheal urethritis with these antibiotics, 206 patients were given triacetyloleandomycin, the last 70 being alternated with 70 patients receiving erythromycin stearate and 65 patients receiving phenoxymethyl penicillin.

One hundred ninety-two patients (approximately 94 per cent) of the 206 treated with triacetyloleandomycin were considered to have been cured. Sixty patients (approximately 86 per cent) were cured by erythromycin, and 55 patients (approximately 84 per cent) of those receiving phenoxymethyl penicillin were cured.

#### TOXIC REACTIONS

None of these patients who were treated with the above oral dosages of triacetyloleandomycin, erythromycin stearate, and phenoxymethyl penicillin experienced any adverse reactions as a result of having received such antibiotic therapy.

#### DISCUSSION

These therapeutic results—94 per cent cures obtained with triacetyloleandomycin—seem to indicate that this new antibiotic is superior to erythromycin and phenoxymethyl penicillin—86 and 84 per cent cures, respectively, for the oral treatment of acute gonococcal urethritis.

This superior effectiveness of triacetyloleandomycin in the treatment of acute gonococcal urethritis might be explained partly by the higher blood levels and consequently greater urinary excretion (23 per cent during 24 hours) attained with this new antibiotic, especially when these figures are compared with those of its predecessor, oleandomycin, and those of erythromycin.

# Antibiotic Management of Acute Infections in the Obstetric Patient

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The treatment of acute infections related with pregnancy, in any of its periods, or with postpartum, in spite of the precautionary use of antibiotics, is becoming a matter of concern, on account of the appearance of microbial strains naturally resistant or that have become resistant to those antibiotics of more common usage.

Among these infections, acute puerperal mastitis as well as acute pyelonephritis, are the two clinical entities that are still seen frequently in obstetric practice and that present therapeutic problems, due to the fact that penicillin and streptomycin have recently proved ineffective, on account of the development of resistance.

These facts induced us to perform clinical trials with the antibiotic combination of tetracycline and oleandomycin,\* in order to determine its effectiveness in those infections associated with puerperium.

## MATERIAL AND METHODS

Thirty-two patients were involved in these trials; 20 with acute mastitis due to *Staphylococcus aureus* and 12 with acute pyelonephritis, mostly of medium intensity which, as a rule, is resistant to penicillin, streptomycin, and sulfonamides. For this study we selected patients with mastitis who were in the presuppurative period. It is a known fact that in this type of infection the causative organism usually is the *Staph. aureus*, which, in a great percentage of cases, is already resistant to the more common antibiotics, specifically, to penicillin and streptomycin.

Twenty patients with acute mastitis due to *Staph. aureus* were involved in this series. In several of these cases the mother was still nursing the child. In these therapeutic trials, the drug was administered orally in daily dosages ranging from 1.25 to 2 Gm. in divided doses. The duration of therapy generally varied from 3 to 7 days.

## RESULTS

The most important information gathered from these two groups of patients is listed in tables I and II, which refer to acute puerperal mastitis and related ailments and acute pyelonephritis in the obstetric patients, respectively.

*Acute Puerperal Mastitis.* In the cases of acute mastitis, the results obtained with the antibiotic combination were more favorable compared to those cases treated previously with other remedies. In spite of treatment, 6 of the 20 patients with mastitis developed suppuration. We must add, however, that in 1 patient the focus had already formed when our treatment was started, and it was necessary to drain surgically. In this same patient, however, a second focus of mastitis that had appeared simultaneously with the first one, but in another sector of the breast, healed perfectly without suppuration (patient 4).

Patient 6 developed the first focus of mastitis one week after delivery and was

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This work was carried out at the Obstetric and Gynecologic Clinic directed by Professor J. J. Crottogini.

\* The trade name of Chas. Pfizer & Co. for the combination of oleandomycin and tetracycline is Signemycin.

TABLE I  
*Acute Puerperal Mastitis*

Patient no.	Diagnosis	Results*	Previous Treatment	Remarks
1	Bilateral acute mastitis	Poor	Sulfonamides, vaccine	Development of suppuration
2	Acute mastitis	Excellent	None	
3	Mastitis	Poor	None	Suppuration
4	Mastitis (bilateral)	Good	Penicillin plus streptomycin 800,000	Healing of one; suppuration and drainage of the other
5	Mastitis	Excellent	Penicillin-streptomycin	Without suppuration
6	Acute mastitis	Excellent	Penicillin-streptomycin	
7	Acute mastitis	Excellent		Cured without suppuration
8	Acute mastitis	Excellent		Cured without suppuration
9	Acute mastitis	Excellent		Cured without suppuration
10	Acute mastitis	Excellent		Cured without suppuration
11	Nipple fissure	Good		The adenopathy persisted
12	Nipple fissure	Excellent		
13	Acute mastitis	Excellent	Penicillin-streptomycin	
14	Acute mastitis	Excellent		
15	Acute mastitis	Excellent	Penicillin-streptomycin	
16	Acute mastitis	Excellent		
17	Acute mastitis	Good		Suppuration
18	Acute mastitis	Excellent	None	
19	Acute mastitis	Poor	None	Suppuration
20	Acute mastitis	Excellent	None	

\* Excellent, when suppuration or surgical drainage was avoided; good, when, even though surgical drainage was not performed, suppuration or regional adenopathy appeared in spite of treatment; poor, when suppuration appeared, making drainage necessary.

treated with a combination of penicillin and streptomycin, but suppuration developed requiring drainage. After 10 days, a new focus appeared that was treated daily with 1.50 Gm. of the oleandomycin-tetracycline combination for five days, healing before reaching the suppuration stage.

Even though our experience is limited, we can state that the suppression of the abscess formation stage constitutes the real clinical test of the effectiveness of the therapy employed. This suppression was observed in 80 per cent of the patients treated with the oleandomycin-tetracycline combination. This result was partial in 1 patient, as one of the two foci had to be drained surgically.

TABLE II  
*Acute Pyelonephritis*

Patient no.	Symptomatology	Results	Previous Treatment	Remarks
1	Dysuria and pyuria	Excellent	None	
2	Medium intensity	Good	None	The temperature became normal after 48 hours of treatment
3	Lumbalgia, dysuria, fever	Excellent	None	
4	Cystitis, pyelitis, pre-eclampsia	Excellent	None	
5	Cystitis, pyuria	Excellent	None	
6	Fever, intense aches	Good	None	
7	Fever	Excellent	None	Eight months gravidity
8	Lumbar ache, pyuria, dysuria, fever	Excellent	None	Primípara
9	Late gravidic toxemia	Poor	None	The dysuria continued; probably wasn't a real pyelonephritis
10	Fever	Excellent	None	
11	Pollakiuria, dysuria, apyretic	Poor	None	The treatment was continued with sulfonamides
12	Pyuria	Excellent	Penicillin plus streptomycin	

It can be said that therapy failed in 15 per cent of the patients, as healing was not obtained in the presuppurative stage, and the abscess had to be drained surgically. We must bear in mind that, notwithstanding this development, the limitation of the infectious focus and the complete lack of complications was undoubtedly due to the beneficial action of the antibiotic therapy employed.

*Pyelonephritis.* The favorable response obtained in all the cases of authentic pyelonephritis (confirmed) treated with oleandomycin-tetracycline, led us to make, in doubtful cases, an exact diagnosis of the illness, discarding those clinical patterns with lumbago and pyuria, which, upon a bacteriological examination, had colibacillus simulating pyelonephritis.

Based on the real anatomico-clinical substratum of the disease, many diagnoses of pyelonephritis were erroneous, and for said reason the remedy did not act in these patients.

#### TOLERANCE

Tolerance was perfect; no side effects or inconveniences were observed that could be attributed to the treatment, and it was never necessary to suspend the drug.

#### SUMMARY

Acute puerperal mastitis as well as acute pyelonephritis are two clinical entities that are still seen with some frequency in obstetric practice. Such cases frequently present difficult therapeutic problems, particularly when the pathogenic organism demonstrates resistance to commonly employed antibacterial agents. In our experience, penicillin and streptomycin have recently proved disappointing due to the development of resistance. For this reason, clinical trial was undertaken with the antibiotic combination of tetracycline and oleandomycin to determine its effectiveness in infections associated with the puerperium. Thirty-two patients were involved in these trials: 20 with acute mastitis due to *Staphylococcus aureus* and 12 with acute pyelonephritis. All patients received tetracycline plus oleandomycin in daily dosages ranging from 1.25 to 2.0 Gm. Duration of therapy generally varied from 3 to 6 days, with 3 patients receiving medication for seven days. The efficacy of the antibiotic combination was evaluated on the basis of clinical experience with similar cases. The pathology of acute puerperal mastitis is well known, the condition generally proceeding to abscess formation. With the treatment employed in this series, abscess formation was promptly forestalled in 16 of the 20 patients. The remaining patients responded following the combined surgical intervention and antibiotic therapy. The cases of acute pyelonephritis uniformly responded, except where anatomic malformation was present. In the dosage employed, no side effects were noted in any of these 32 patients.

#### BIBLIOGRAPHY

1. QUARTI, M.: Efficacy of the association of oleandomycin and tetracycline in staphylococcal infections, *Aggiornamento Pediátrico* 8:813-820, 1957.
2. LACAILLE, R. A., AND PRIGOT, A.: Combinations of oleandomycin with oxytetracycline and tetracycline in soft-tissue infections. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 67.
3. REVELLI, E., AND DURANDO, C.: Treatment of the specific inflammatory component of tuberculosis of the female genital organs, *Minerva med.* 48:2658, Aug. 25, 1957.

# The Use of Triacetyloleandomycin in Chronic Infectious Asthma

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Patients with chronic asthma usually have an infectious component.<sup>1-4</sup> The signs of cough, wheezing, and attacks of dyspnea are always associated with the amount and type of sputum produced. It is not readily agreed as to how bacteria act in the allergic asthmatic patient, but it is apparent that infection may initiate or precipitate an attack and contribute to the type and amount of sputum. Many investigators have attempted to correlate the bacteriology of the sputum with the type and severity of the patient's asthma, but without definite conclusions.<sup>5</sup> This may be because of the difficulties in collecting specimens of tracheobronchial secretions without contamination from organisms of the pharynx and upper respiratory tract.<sup>6</sup>

Many antibiotics and chemotherapeutic agents have been used in the management of infectious asthma. Blatt,<sup>7</sup> Radnote-Recht,<sup>8</sup> and others used several broad-spectrum antibiotics in this condition with variable results. We have used many different antibiotics in these cases but have found a high incidence of undesirable side effects and poor clinical response.

Triacetyloleandomycin (Tao\*) is a chemical derivative prepared by the acetylation of three free hydroxyl groups in the parent antibiotic, oleandomycin.<sup>9,10</sup> Such modification has resulted in several advantages over the parent compound, including greater stability in the presence of gastric acid, lack of objectionable taste, and rapid attainment of high serum levels. *Streptococcus pyogenes*, *Diplococcus pneumoniae*, *Corynebacterium diphtheriae*, and *Hemophilus influenzae*, potential pathogens in the respiratory tract, are sensitive to triacetyloleandomycin in vitro.<sup>11-13</sup> Seventy-three per cent of *Staph. aureus* resistant to erythromycin were sensitive to triacetyloleandomycin.<sup>14</sup>

This combination of advantages seemed to make it desirable as a therapeutic agent in the treatment of infectious asthma, particularly in those cases in which long-term antibiotic therapy was required.

## MATERIALS AND METHODS

Patients with chronic asthma were selected from among the following five groups: (1) emphysema with infection; (2) chronic bronchitis; (3) bronchiectasis; (4) allergic rhinitis, sinusitis, and polyps; and (5) asthma superimposed on an acute allergic episode and/or acute upper respiratory infection.

The ages of the patients ranged from 13 to 70 years old. Each was given one 250 mg. capsule of triacetyloleandomycin four times daily for one week, then one capsule three times daily for another week, and, finally, in some cases as little as one capsule per day, depending on need.

Sputum samples were collected immediately prior to therapy in sterile screw cap jars and at intervals of seven days thereafter, while the patient was on the drug. The sputa were cultured on blood and chocolate agar under 10 per cent carbon

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\* The trade name of the J. B. Roerig & Co. Division, Chas. Pfizer & Co., for triacetyloleandomycin is Tao.

TABLE I  
*Predominating Flora of Potentially Pathogenic Bacteria\**  
*in Sputum of 44 Patients with Bronchial Asthma*

Organism	Before therapy		After therapy	
	No.	Per cent	No.	Per cent
<i>Streptococcus pyogenes</i>	8	17	5	11
Pneumococci	32	73	22	50
Hemolytic <i>Staphylococcus aureus</i> , coagulase-positive	18	41	15	34
<i>Hemophilus influenzae</i>	11	25	13	29
Coliforms; <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , paracolon	19	43	15	34
<i>Proteus</i>	6	14	7	16
<i>Pseudomonas aeruginosa</i>	11	25	13	29
<i>Candida albicans</i>	22	50	29	66

\* Omitted from the table are the following organisms considered to be primarily saprophytic: Alpha- and gamma-streptococci, coagulase-negative *Staphylococcus*, diphtheroids, neisseriae, *Hemophilus hemolyticus*, and saprophytic yeasts.

dioxide and in thioglycollate broth. Organisms were identified by the usual bacteriological techniques.

All isolates of gram-positive cocci and of *H. influenzae* from sputa collected before and after therapy were tested for susceptibility to oleandomycin by the disc method. This was done to determine if there was any increase in the number of strains resistant to this drug. Other species were tested for antibiotic susceptibility, as indicated.

#### RESULTS

*Bacteriological.* There were no profound changes in the bacterial flora noted on cultures taken before and after therapy (table I). No records of the occurrence of organisms, such as *Streptococcus viridans*, nonhemolytic staphylococci, *Neisseria*, and the like, were kept in this series. These bacteria are commonly considered as normal inhabitants of the nasopharynx, rather than the bronchi or lungs, and have a very low index of pathogenicity. It is possible, however, that their numbers, rather than the type, may be associated with the infectious component in these patients.

All strains of *Str. pyogenes* and pneumococci were susceptible to oleandomycin, in the pretreatment and post-treatment specimens. All but one strain of *Staph. aureus* was sensitive in the first and second specimens. Seven of 11 (64 per cent) of the strains of *H. influenzae* were sensitive in the first, and 8 of 13 (62 per cent)

TABLE II  
*Type of Asthma and Clinical Results Obtained after Treatment with Triacetyloleandomycin*

Diagnosis	No. of patients	Improved*		Not improved	
		No.	Per cent	No.	Per cent
Emphysema with infection	6	5	83	1	16
Chronic bronchitis	21	19	90	2	10
Chronic bronchiectasis	3	2	66	1	33
Sinusitis or rhinitis	7	6	86	1	14
Allergic asthma with infection	7	6	86	1	14
	44	38	84	6	16

\* Lowered requirements for iodides, bronchodilators, and steroids; reduction in cough, wheezing and sputum; and increased pulmonary function.

in the second specimens. Thus, there was no evidence of increased resistance to oleandomycin in these organisms acquired during treatment with triacetyloleandomycin.

*Clinical.* The main effect noted was a change in the type, amount, and consistency of the sputum, with a consequent gratifying reduction in the signs of cough, expectoration, and wheezing in 38 of 44 patients (84 per cent). The amount of iodides, bronchodilators, steroids, antibiotics, and other therapeutic agents required was markedly reduced, and the patients' feeling of well-being concomitantly improved. In no case was it necessary to discontinue the drug because of any untoward reaction. The improvement in the patients' conditions could not be correlated with the bacteriological findings directly (table II).

#### CASE REPORTS

*Case 1.* S. K., a 44 year old man, had a history of long-standing cough, wheezing, dyspnea and copious mucopurulent sputum, as well as personal and family history of allergies. He was treated specifically as well as nonspecifically, with a wide variety of agents. Roentgenogram and bronchoscopy were positive for bilateral bronchiectasis, involving two lobes on the right side. Therapeutic bronchoscopy was necessary every two to four weeks. Since being placed on triacetyloleandomycin, bronchoscopy has no longer been necessary, and the sputum has been markedly reduced in consistency.

*Case 2.* H. R., a 57 year old man, had a history of chronic cough, wheezing, and difficulty breathing, especially after retiring at night. Sputum was heavy, thick, copious, and mucopurulent. Roentgenogram was positive for maxillary and ethmoid sinus infection. The patient was treated with broad-spectrum antibiotics, iodides, bronchodilators, and steroids. One week after triacetyloleandomycin was started, the consistency and amount of sputum changed, and the need for bronchodilators diminished.

*Case 3.* P. C., a 35 year old man, had a long history of dyspnea, cough, and wheezing, and several operations for polyps and deviated septum. There was a history of allergy in the family, as well as in the patient. Skin tests were positive for molds, dust, and foods. Avoidance, elimination and specific therapy were helpful, but wheezing continued and sputum remained mucopurulent. One week after triacetyloleandomycin was started, sputum became less purulent, and wheezing diminished. Asthma was completely controlled on maintenance dose of triacetyloleandomycin.

*Case 4.* J. S., a 60 year old woman, had a 20 year history of chronic bronchitis and asthma. Sputum was copious and mucopurulent. Roentgenogram gave evidence of bronchitis. There was no history of personal or familial allergy. The patient had been treated with broad-spectrum antibiotics, iodides, bronchodilators, steroids, detergents, and enzymes. Triacetyloleandomycin was added to other therapeutic measures, and wheezing diminished, need for other drugs lessened, and respiratory function improved. Patient is still on maintenance dose.

*Case 5.* J. D., a 13 year old boy, had seasonal and perennial symptoms of bronchial asthma, often initiated by upper respiratory infections. Skin tests were positive for foods, molds, dust, and pollens. The patient was treated specifically with good results but had bouts of upper respiratory infection, with continuous wheezing and asthma. Antibiotics and chemotherapeutic agents were used for the acute attacks. The patient was then placed on triacetyloleandomycin and maintained on small dose. No asthma, wheezing or upper respiratory infection have occurred for the past four months.

#### DISCUSSION

It has been noted that whenever the sputum is thick, tenacious, copious, and mucopurulent, the degree of coughing, wheezing, and attacks of so-called "asthma" are increased. When the consistency of the sputum is changed to thin and watery and the amount is reduced, the frequency of attacks of asthma is diminished. When iodides, bronchodilators, steroids, enzymes, and detergents are given, improvement

is noted, but the improvement is generally short-lived, and the condition usually recurs with undiminished severity. We have found that the administration of triacetyloleandomycin caused a rapid change in the amount and type of sputum from purulent to watery and a marked diminution in quantity. The changes in the bacterial flora could not be correlated with the changes in the nature of the sputum. This may be inherent in the method utilized in the collection of the sputum. Perhaps if sputa had been taken by bronchial catheter, some marked changes might have become apparent. Studies have shown<sup>6</sup> that ordinary methods of collection result in rapid contamination with other organisms of the upper respiratory tract. Furthermore, it is possible that the clinical improvement noted may be due to the diminution of the number, rather than the types of bacteria, thereby reducing the amount of allergenic material bathing the tracheal bronchi and alveoli and in this way reducing the mucopurulent nature of the sputum.

Also noteworthy in this study is the fact that no evidence was elicited of any increase of resistance to triacetyloleandomycin by any other bacteria tested nor any evidence of superinfection.

It is quite possible that other antibiotics may give results so satisfactory as or superior to triacetyloleandomycin in the management of infectious asthma, provided that they can be safely given for a long period of time, if necessary. Our main emphasis in this study is that the judicious use of the proper antibiotic can be of distinct value in the therapy of infectious asthma.

#### SUMMARY

The value of triacetyloleandomycin in the management of infectious asthma is discussed.

Triacetyloleandomycin in small doses afforded improvement in 84 per cent of cases of various types of chronic infectious asthma. The role of bacteria and the rationale for the use of antibiotics in this condition is briefly discussed.

#### BIBLIOGRAPHY

1. CHOBOT, R.: Asthma in children, *Am. J. Dis. Child.* 45:25, 1933.
2. CHOBOT, R.; UVITSKY, I., AND DUNDY, H.: Relationship of the etiological factors in asthma in infants and children, *J. Allergy* 22:106, 1951.
3. COOKE, R.: *Allergy in Theory and Practice*, Philadelphia, W. B. Saunders & Co., 1947.
4. BIVINGS, L.: Asthmatic bronchitis following chronic upper respiratory infection, *J.A.M.A.* 115:1434, 1940.
5. UFFORD, V. J., Ed.: *Therapy in Bronchial Asthma*, Leiden, A. J. Kroese, 1956.
6. BERGMAN, S., AND COLDLAHL, H.: Sputum diagnosis in asthma bronchiale with special reference to the occurrence of a complicating bronchial infection, *Acta Allergica* 10:63, 1956.
7. BLATT, H.: Determination of dosage for tetracycline and oxytetracycline when used in conjunction with bacterial filtrates for desensitization, *AM & CT* 5:19, 1958.
8. RADNOTE-RECHT, I.: The role of catarrhal infection of the respiratory tract and of bacterial allergy in the pathogenesis and treatment of bronchial asthma in children, *Monatschr. f. Kinderk.* 104:318, 1956.
9. CELMER, W. D., AND HOCHSTEIN, F. A.: Oleandomycin. Mono- and diacetate esters. Preparation and characterization. Presented at the 133rd meeting of the American Chemical Society, San Francisco, Calif., April 16, 1958.
10. CELMER, W. D.; ELS, H., AND MURAI, K.: Oleandomycin derivatives. Preparation and characterization. In: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 476.
11. SHUBIN, H.; DUMAS, K., AND SOKMENSUER, A.: Clinical and laboratory studies on a new derivative of oleandomycin. In: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 679.
12. ENGLISH, A. R.: Oleandomycin: Cross-resistance studies with clinical isolates of *Micrococcus pyogenes* var. *aureus*. In: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 756.
13. WENNERSTEN, J. R.: Effectiveness of triacetyloleandomycin in a wide variety of infections, *AM & CT* 5:527, 1958.
14. ENGLISH, A. R., AND FINK, F. C.: Indications for the usefulness of triacetyloleandomycin in epidemic staphylococcal infections, *Antib. & Chemo.* 8:420, 1958.

Triacetyloleandomycin (Tao\*) is a novel therapeutically effective antibiotic substance of considerable interest<sup>1-5</sup> (fig. 1). Chemically, triacetyloleandomycin represents a variant of oleandomycin in which all available free hydroxyl groups have been modified. Marked physical, chemical, and biological changes resulted from the conversion of oleandomycin to triacetyloleandomycin. Increased stability, decreased water solubility, and enhancement of taste properties have been observed.<sup>1</sup> Furthermore, the drug exhibited the unusual ability to promote higher antibiotic levels in body fluids, following ingestion by human beings.<sup>2</sup> Characteristics of the pertinent model compounds, tripropionyleandomycin (TPO), tributyrileandomycin (TBO) the diacetyloleandomycins (2,3-DAO, 1,3-DAO, 1,2-DAO), and the monoacetyloleandomycins (3-MAO, 2-MAO, 1-MAO), have been determined (fig. 1). Notable correlations of biological and chemical properties with particular acylation sites and acyl species have been observed. Evidence has been provided that many of the properties of triacetyloleandomycin are resultants of individual substituent effects.

## CHEMICAL AND PHYSICAL ASPECTS

The acetyl substituents of triacetyloleandomycin reside in diverse structural moieties (fig. 1). Advantage was taken of differential reaction rates at the various acylation sites in the preparation of all of the theoretically possible partial acetyl esters of oleandomycin.<sup>6</sup> In general, acetyl groups were introduced according to the relative reactivities  $R_1 > R_2 > R_3$  (fig. 2), which afforded the step-wise preparative scheme: oleandomycin  $\longrightarrow$  1-MAO  $\longrightarrow$  1,2-DAO  $\longrightarrow$  triacetyloleandomycin.

Triacetyloleandomycin readily dissolved in aqueous acid, retaining its biological potency and identity. Removal of acetyl substituents by saponification or uncatalyzed methanolysis proceeded in the same order as they were introduced, i.e.,  $R_1 > R_2 > R_3$ , which allowed the preparation of partially acetylated derivatives inaccessible by direct acetylation: triacetyloleandomycin  $\longrightarrow$  2,3-DAO  $\longrightarrow$  3-MAO. An alternate deacetylation sequence,  $R_2 > R_1 > R_3$ , was observed, utilizing alkali-catalyzed methanolysis conditions: triacetyloleandomycin  $\longrightarrow$  1,3-DAO  $\longrightarrow$  3-MAO. The remaining acetyl substituent in 3-MAO was difficult to remove by chemical means; in terms of relative retention of antimicrobial activity in alkaline solutions, 3-MAO was more stable than oleandomycin. Thus, the stability characteristics of triacetyloleandomycin are reflected by its inherent chemical nature as well as physical properties, such as well defined crystallinity, relatively high melting point, lower water solubility, and nonhygroscopicity.

The diacetyl ester 1,2-DAO, an intermediate in the preparation of triacetyloleandomycin, could be alternatively deacetylated according to the schemes  $R_1 > R_2$  or  $R_2 > R_1$ , depending on reaction conditions (see preceding). The intermediate

\* The trade name of the J. B. Roerig & Co. Division, Chas. Pfizer & Co. for triacetyloleandomycin is Tao.

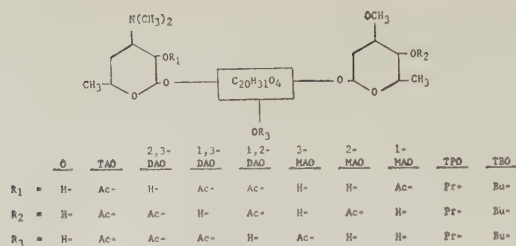


FIG. 1. Structural differentiation of oleandomycin (O), triacetyloleandomycin (TAO), isomeric diacetyloleandomycins (DAO), isomeric monoacetyloleandomycins (MAO), tripropionyleandomycin (TPO) and tributyrileandomycin (TBO) are shown, Ac-:  $\text{CH}_3\text{CO}-$ ; Pr-:  $\text{CH}_3\text{CH}_2\text{CO}-$ ; Bu-:  $\text{CH}_3\text{CH}_2\text{CH}_2\text{CO}-$ .

monoesters 2-MAO (available by deacetylation of 1,2-DAO) and 1-MAO were notably more readily reconverted to oleandomycin than 3-MAO by chemical deacetylation reactions.

Distinguishing chemical and physical properties of triacetyloleandomycin and pertinent model compounds are summarized in table I. Low basicity (about  $pK_a$  6.6), violet Keller-Kiliani test, and positive rotary dispersion Cotton effect are diagnostic means for detecting acyl substituents on the desosamine, *L*-oleandrose, and oleandolide moieties, respectively. Solvent-aqueous distribution characteristics, exhibited by triacetyloleandomycin, oleandomycin, and the six partially acetylated derivatives (fig. 3), permitted their effective separations for diagnostic and preparative purposes. Similar differential patterns on paper chromatographs have been observed by Lees et al.<sup>7</sup>

#### BIOLOGICAL ASPECTS

The *in vitro* antimicrobial activities of triacetyloleandomycin and related compounds are expressed in fig. 4. The lower *in vitro* potency of triacetyloleandomycin is also exhibited by derivatives that bear a substituent of the *L*-oleandrose ( $R_2$ ) moiety (2,3-DAO, 1,2-DAO, 2-MAO, TPO, TBO). However, *in vitro* potencies showed no correlation with relative *in vivo* absorption effects (figs. 5 and 6). The high antibiotic serum levels and antibiotic recoveries in urine after the ingestion of the drug by human beings<sup>2</sup> were also observed with the specific derivatives 1,3-DAO, 1,2-DAO, 1-MAO, and TPO.<sup>8,9</sup> These compounds, like triacetyloleandomycin are characterized by desosamine substitution. However, the nature of the substituent appears to be critical, since TBO, which fits into the desosamine-substituted category, was not absorbed well. Thus, the enhanced absorption effects observed with triacetyloleandomycin and related substances cannot be ascribed to

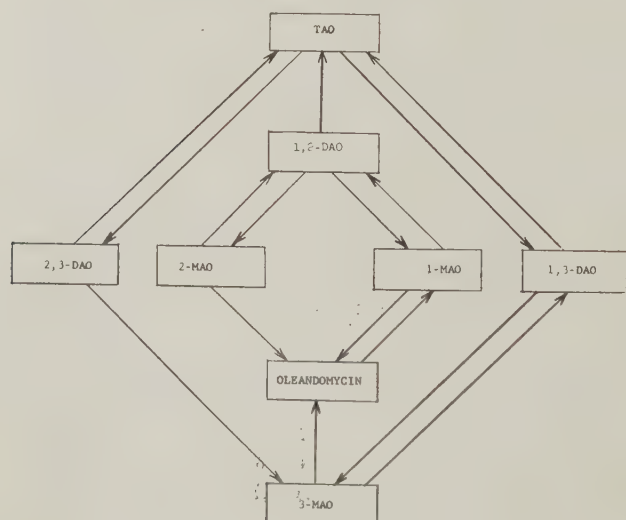


FIG. 2. Chemical interconversions are illustrated. DAO, isomeric diacetyloleandomycins; TAO, triacetyloleandomycin; MAO, isomeric monoacetyloleandomycins.

TABLE I  
Identification Properties

Compound	Melting point, C.	Acyl no.	pK <sub>a</sub>	K.K. test†	$\alpha$ 325 m $\mu$ ‡
Oleandomycin	110	0.00	8.5	Red	—
TBO	114	2.90	6.6	Violet	+
TPO	158	2.82	6.6	Violet	+
Triacetyloleandomycin	177	3.00	6.6	Violet	+
2,3-DAO	182	1.98	7.7	Violet	+
1,3-DAO	162	2.00	6.6	Red	+
1,2-DAO	*	2.04	6.6	Violet	—
3-MAO	182	1.04	8.0	Red	+
2-MAO	*	1.00	8.0	Violet	—
1-MAO	*	1.08	6.7	Red	—

\* Amorphous solid.

† Keller-Kiliani color test.

‡ — = optical rotatory dispersion cotton effect trough (levorotatory); + = optical rotatory dispersion cotton effect peak (dextrorotatory).

desosamine substitution (and/or low basicity) *a priori*<sup>10</sup> but rather to a combination of additional factors, including solubility and stability in the digestive tract as well as substituent influences on metabolic processes within the circulatory system, tissues, and various organs.<sup>11</sup>

Insight to the fate of orally administered triacetyloleandomycin was gained by a study of its metabolites. A variety of microbiologically active substances were de-

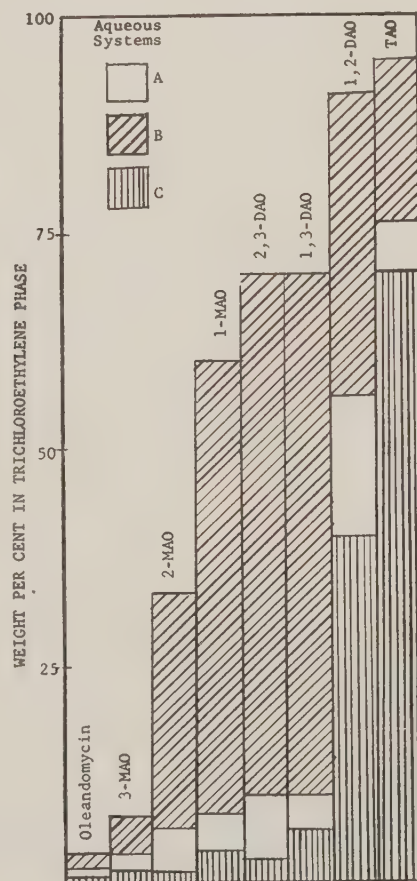


FIG. 3. Comparative distribution patterns of antibiotic substances exhibited between trichloroethylene and various aqueous systems are given. System A, 3.5 *N* aqueous acetic acid; System B, system A adjusted to pH 4 with 10 *N* sodium hydroxide; System C, 1 *N* aqueous ascorbic acid. DAO, isomeric diacetyloleandomycins; TAO, triacetyloleandomycin; MAO, isomeric monoacetyloleandomycins.

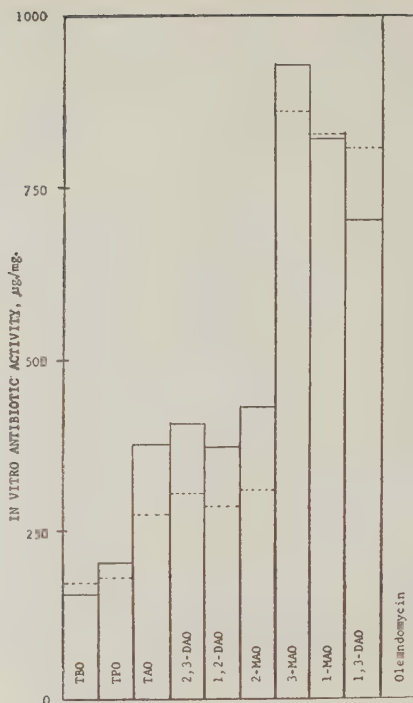


FIG. 4. Comparative antibiotic activities are expressed in terms of oleandomycin base. --- vs. *Staphylococcus aureus* (turbidimetric); — vs. *Sarcina lutea* (plate diffusion). DAO, isomeric diacetyloleandomycins; TAO; triacetyloleandomycin; MAO, isomeric monoacetyloleandomycins.

tected in urine following ingestion of the drug and other oleandomycin acetate esters (table II).<sup>7</sup> The extent and nature of the in vivo hydrolytic patterns exhibited by the drug and its model compounds were surprisingly different from predictions based solely on chemical deacetylation analogies. It is apparent that the drug may follow a variety of in vivo hydrolytic pathways (fig. 7) to account for the observed metabolic products.

#### MATERIALS AND METHODS

**Oleandomycin Esters.** The acetate esters utilized in this study have been described previously.<sup>1, 6</sup> The homologous triesters, TPO and TBO, were prepared by the acylation of oleandomycin with propionic anhydride and butyric anhydride, respectively, according to the procedure described for triacetyloleandomycin.<sup>1</sup> Crystallization of TPO from ether-hexane gave colorless needles, with melting point at 158 C. Calculated for  $C_{44}H_{73}NO_{15}$ : propionyl, 20.0 per cent; found, 18.8 per cent. Crystallization of TBO from hexane afforded colorless prisms, with melting point at

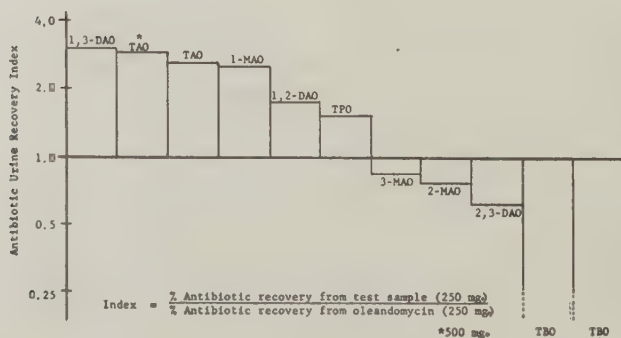
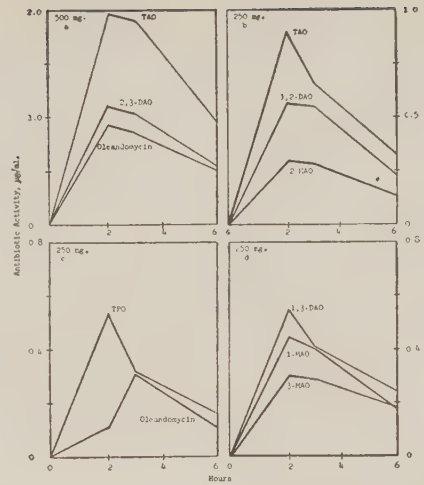


FIG. 5. Comparative antibiotic urine recovery indexes (oleandomycin = 1.0) are illustrated. Each index is based on composite 8 hour urines following single oral doses in crossover experiments with human subjects.<sup>2, 8</sup> DAO, isomeric diacetyloleandomycins; TAO, triacetyloleandomycin; MAO, isomeric monoacetyloleandomycins.

FIG. 6. Given are comparative average antibiotic concentrations in human blood serum following cited single oral doses. a, 30 subject crossover study;<sup>2</sup> b, c, and d, 10 subjects. Antibiotic activity is expressed in terms of oleandomycin base. DAO, isomeric diacetyloleandomycins; TAO, triacetyloleandomycin; MAO, isomeric monoacetyloleandomycins; TPO, tripropionyleandomycin.



114 C. Calculated for  $C_{47}H_{79}NO_{15}$ : butyryl, 23.7 per cent; found, 22.8 per cent. Identification characteristics of the test compounds are reviewed in table I.

*Solvent Distribution Studies.* A 2.0 Gm. sample of the test compound was added to a two phase system containing 40.0 ml. of trichloroethylene and 40.0 ml. of an aqueous acidic solution (dilute acetic acid, pH 4 acetate buffer, or dilute ascorbic acid). After the solid dissolved, the two phases were shaken for several minutes and allowed to settle. The trichloroethylene phase was then collected, evaporated, and the dried residue was weighed. The relative distribution properties of the various compounds are expressed in figure 3.

*Microbiological Assays.* The esters were assayed by plate diffusion and turbidimetric methods, employing the response curve obtained with oleandomycin test organisms. The qualitative nature of the response curves exhibited by the various esters was indistinguishable from that of the reference. The in vitro potencies, in terms of oleandomycin activity, are illustrated in figure 4.

*Urine and Blood Samples.* Specimens were obtained from normal young men at various intervals after ingestion of the pure test compounds\* enclosed in gelatin capsules. Urine pooled after eight hours was stirred with an equal volume of trichloroethylene and adjusted to pH 9 with normal sodium hydroxide. The solvent

TABLE II  
Metabolic Fate of Ingested Antibiotic Substances

Compound ingested	Antibiotics identified in blood serum and urine*
Oleandomycin	Oleandomycin
Triacetyloleandomycin	Oleandomycin, 3-MAO (major) 1-MAO, 1,3-DAO (intermediate) 2-MAO (minor) 2,3-DAO (trace)
2,3-DAO	Oleandomycin, 2,3-DAO, 3-MAO, 2-MAO
1,3-DAO	Oleandomycin, 1,3-DAO, 3-MAO, 1-MAO
1,2-DAO	Oleandomycin, 1-MAO, 2-MAO, 1,2-DAO (trace)
3-MAO	3-MAO, oleandomycin (minor)
2-MAO	Oleandomycin, 2-MAO
1-MAO	Oleandomycin, 1-MAO

\* An unidentified antibiotic substance was detected in blood serum but not in urine.<sup>7</sup>

\* All of the esters exhibited an LD<sub>50</sub> of 5000 mg./Kg. or greater after oral administration to rats.<sup>12, 13</sup>

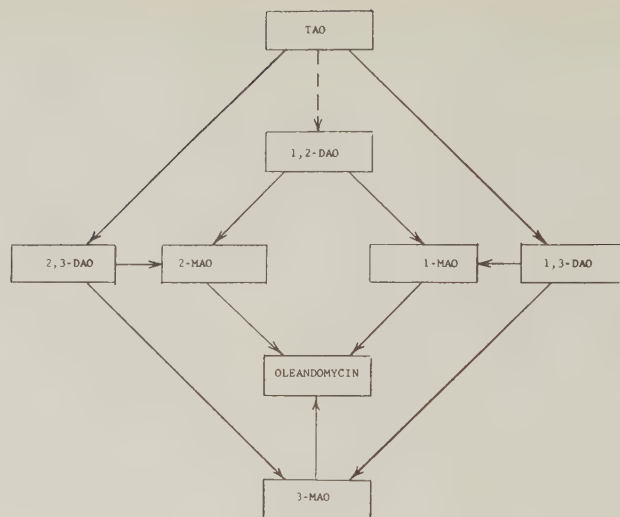


FIG. 7. In vivo deacetylation pathways are shown. DAO, isomeric diacetyloleandomycins; TAO, triacetyloleandomycin; MAO, isomeric monoacetyloleandomycins.

phase was collected, filtered through cotton to remove entrained moisture, and the filtrate was evaporated in vacuo. The dried residue was then subjected to paper chromatography, and the resolved microbiologically active components were detected by bioautographs. The antibiotic substances identified in urine for each ingested ester are compiled in table II. Estimates of the relative proportions of each component were based on the relative sizes of inhibition zones.<sup>7</sup>

Detail of testing procedures, involving groups of subjects, designed to obtain significant quantitative measurements of antibiotic excretion in urine, will be presented in a forthcoming publication.<sup>8</sup> Urine antibiotic recovery indexes for the various ingested esters are reviewed in figure 5.

Blood samples were processed to obtain the serum fractions, which were then assayed microbiologically. The antibiotic levels observed at various time intervals by Dumas and Kersey<sup>9</sup> are shown in figure 6. Pooled serum samples were extracted twice with three volumes of trichloroethylene. The combined solvent extracts were filtered through cotton, and the filtrate was evaporated to dryness, in vacuo. The residue was examined by paper chromatography as described previously (table II).

**Stability Studies.**<sup>14</sup> Samples of a pH 2.5 aqueous solution of triacetyloleandomycin (50 mg./ml.) were subjected to 25 and 37C. temperatures, and were assayed periodically over a period of three weeks. No significant change in microbiological potency or composition was observed at either temperature throughout the study. Aqueous oleandomycin phosphate solutions, after three weeks of similar testing, showed no appreciable change at 25C., whereas at 37C. a 25 per cent loss of potency was measured. Oleandomycin and 3-MAO solutions compared at pH 9.0 showed 50 per cent and less than 10 per cent activity losses, respectively, after three weeks at 25C.

#### SUMMARY AND CONCLUSIONS

1. Crystalline triacetyloleandomycin has been compared with homologous triesters and all of the theoretically possible partially acetylated variants of oleandomycin. Correlations of biological and chemical properties of the drug with certain structural features have been presented.

2. The high antibiotic absorption effects noted following the ingestion of tri-

acetyloleandomycin by human beings have been particularly associated with the acetyl substituent on the desosamine ( $R_1$ ) moiety. This phenomenon was limited to specific acyl species.

3. The degree of in vitro antibiotic activity exhibited by triacetyloleandomycin has been attributed chiefly to the acetyl substituent on the *L*-oleandrose ( $R_2$ ) structural moiety.

4. Latent alkaline stability of triacetyloleandomycin has been correlated with the acetyl substituent on the oleandolide ( $R_3$ ) structural nucleus.

5. The concurrent influence of all three acetyl substituents on the solvent distribution and other physical properties of the drug have been cited.

6. Differential reaction rates have attested the chemical nonequivalence of the diverse substitution sites in the structure of triacetyloleandomycin.

7. Multiple microbiologically active components, identified in human blood serum and urine specimens after ingestion of the drug, have been reconciled with a variety of available metabolic pathways that encompass unique in vivo deacetylation sequences.

8. Further studies on triacetyloleandomycin and related compounds are in progress, in the hope of gaining additional information on the intricate processes that govern the biological and chemical properties of the macrolide antibiotics.

#### ACKNOWLEDGMENTS

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#### BIBLIOGRAPHY

1. CELMER, W. D.; ELS, H., AND MURAI, K.: Oleandomycin derivatives. Preparation and characterization. *In*: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 476.
2. SHUBIN, H.; DUMAS, K. J., AND SOKMENSUER, A.: Laboratory and clinical studies on a new derivative of oleandomycin. *In*: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 679.
3. ENGLISH, A. R., AND FINK, F. C.: Indications for the usefulness of triacetyloleandomycin in epidemic staphylococcal infections, *Antib. & Chemo.* 8:420, 1958.
4. WENNERSTEN, J. R.: Effectiveness of triacetyloleandomycin in a wide variety of infections, *AM & CT* 5:527, 1958.
5. ENGLISH, A. R., AND MCBRIDE, T. J.: Triacetyloleandomycin: Biological studies, *Antib. & Chemo.* 8:429, 1958.
6. CELMER, W. D., AND HOCHSTEIN, F. A.: Oleandomycin mono- and diacetate esters. Preparation and characterization. Abstract of Papers, 133rd Meeting of the American Chemical Society, April, 1958, p. 20M.
7. LEES, T. M.; DE MURIA, P. J., AND BOEGEMANN, W. H.: Paper chromatographic separations of multi-component antibiotic mixtures. *Antib. & Chemo.* In press.
8. FINGER, K., *et al*: Antibiotic urine recovery studies in human subjects. Publication in preparation.
9. DUMAS, K. J., AND KERSEY, R. C.: Private communication.
10. SHANKER, L. S.; TOCCO, D. J.; BRODIE, B. B., AND HOGBEN, C. A. M.: Absorption of drugs from the rat small intestine. *J. Pharmacol. & Exper. Therap.* 123:81, 1958.
11. WELLES, J. S.; ANDERSON, R. C., AND CHEN, K. K.: A metabolite of erythromycin. *In*: Antibiotics Annual 1954-1955, New York, Medical Encyclopedia, Inc., 1955, p. 291.
12. P'AN, S. Y.; DELAHUNT, C. S.; KAISER, J. A., AND BAJEK, E. M.: Triacetyloleandomycin: Acute and chronic toxicity in experimental animals, *Antib. & Chemo.* 8:528, 1958.
13. P'AN, S. Y.: Private communication.
14. HAMMER, H. F.; ARMITAGE, H. R.; ELS, H., AND HOCHSTEIN, F. A.: Private communication.

# Clinical and Laboratory Evaluation of Triacetyloleandomycin in a Variety of Pyogenic Infections

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The triacetyl salt of oleandomycin has been shown to be an effective addition to the antibiotic armamentarium, similar in its spectrum to erythromycin. Triacetyloleandomycin was tested during 1957 and 1958 in a group of individuals hospitalized with a variety of common infections and in several children and adults with furunculosis and their contact carriers of *Staphylococcus aureus* at home.

## MATERIALS AND METHODS

All clinical material for bacteriological analysis was cultured routinely on blood agar, eosin methylene blue agar, *Staphylococcus* 110 agar, Mitis-Salivarius agar, Eugon broth and thioglycollate broth. For final identification of organisms, the biochemical and immunochemical characteristic of each bacterium was determined. The antibiotic profile of each microorganism was determined with the disc method.

Children received orally 40 mg./Kg./day of triacetyloleandomycin in four divided doses. Adults received 500 mg. four times daily, or 2 Gm. daily. Cultures were repeated at the end of treatment and again 4 to 10 days later.

## RESULTS

Of 12 cases of pneumonia, 2 were associated with *Diplococcus pneumoniae*, 10 with *Staph. aureus*, and 1 of the latter also had *Klebsiella* in the pharynx. The results were good in all but 1 in whom recovery was slow. Three cases of chronic pneumonitis were treated: 1 in a newborn infant who probably had congenital pneumonia that did not respond, 1 in a child with cystic fibrosis, and another in a child with familial dysautonomia. The temperature decreased with therapy in the latter 2 patients, but there was no appreciable change in the roentgenograms. One infant, 2 months old, with chronic pulmonary hypertension and congenital heart disease developed pneumonia while on oleandomycin prophylaxis. Group A streptococcal infections were encountered three times; in each instance, clinical response was prompt and the patients free of streptococci within 3 to 4 days.

Recurrent pyogenic skin infections were encountered four times in the hospital. Two patients had good results, 1 seemed much better, and 1 showed no improvement. Five individuals in three families with this disease responded to therapy and all but one, who has just developed recurrences of boils, have not had recurrence in two months following treatment. One adult with multiple furunculosis and acne showed marked improvement and clearing of the coagulase-positive *Staphylococcus* during therapy. Another patient with furunculosis is progressing well under therapy. The rest of the family contacts carrying similar staphylococci in their nasopharynx were cleared of these organisms after four days of triacetyloleandomycin therapy.

During an epidemic of *Staphylococcus* pyoderma, conjunctivitis, and ophthalmitis in a newborn nursery caused by *Staph. aureus* 80/81, 7 carriers of this particular strain among obstetrical and pediatric personnel were cleared of this organism after taking 2 Gm. of triacetyloleandomycin daily for four days.

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TABLE I  
Summary of Clinical Cases

Disease	Total no. seen	Results			Toxicity	Age, years			
		Good	Slow	None		0-5	5-10	10-15	over 15
Pneumonia	12	11	1	0		8	1	1	2
Strep throat	3	3	0	0		0	3	0	0
Pharyngitis and/or tonsillitis	4	4	0	0	Vomiting	3	0	1	0
Peritoneal abscess (postoperative)	1	1	0	0		0	0	0	1
Upper respiratory infection, pneumonitis, bronchitis	7	3	1	3		6	1	0	0
Fever of undetermined origin	2	1	0	1	Hepatitis	0	1	1	0
Cervical adenitis	2	1	1	0	Diarrhea	2	0	0	0
Purulent otitis media	2	2	0	0		1	0	1	0
Furunculosis	4	2	1	1		3	0	0	1
Prophylaxis	2	1	0	1		2	0	0	0
Total	39	29	4	6	3	25	6	4	4
Carriers, <i>Staph. aureus</i> 80/81	7	7	0	0	0	0	0	0	7
Familial furunculosis	15	15	0	0	0	4	3	0	8

Patients with purulent otitis media (2), postoperative peritoneal abscess (1), cervical adenitis (3), and upper respiratory infections (4) responded to therapy. One child with a large fluctuating abscess improved under antibiotic therapy plus aspiration. One of the upper respiratory infections, probably viral, failed to respond, but the *D. pneumoniae* present in the pharynx disappeared with treatment.

Seven of the 62 treated individuals acquired resistant bacteria. The infant with congenital pneumonitis had a VA 4 strain of *Staph. aureus* that was sensitive to oleandomycin prior to treatment and resistant after seven days of therapy. *Hemophilus influenzae* resistant to oleandomycin was encountered along with an oleandomycin-resistant *Staphylococcus* after six days of therapy of tonsillitis in a 3 year old patient with diabetes, whose throat culture yielded *D. pneumoniae* and *Staph. aureus* sensitive to oleandomycin prior to therapy. The other oleandomycin-resistant bacteria were nonpathogenic commensals, such as the *Streptococcus viridans*. A summary of the results appears in table I.

TABLE II  
Sensitivity of Routinely Isolated Bacteria to Oleandomycin

Organism	Total no.	Sensitivity, per cent		
		Sensitive	Slightly sensitive	Resistant
<i>Staphylococcus aureus</i>	1256	71	14	15
<i>Streptococcus</i>				
Group A	67	100	0	0
Group D (enterococcus)	231	80	12	8
<i>Str. viridans</i>	239	92	4	4
Nonhemolytic	97	100	0	0
<i>Diplococcus pneumoniae</i>	113	100	0	0
<i>Hemophilus influenzae</i>	32	25	72	3
<i>Pseudomonas aeruginosa</i>	134	1	0	99
<i>Salmonella</i> sp.	17	0	0	100
<i>Arizona</i> sp.	6	0	0	100
<i>Klebsiella</i> sp.	202	6	5	89
<i>Escherichia coli</i>	309	8	11	81
<i>Escherichia freundii</i>	8	0	12	88
<i>Proteus</i> sp.	137	0	3	97
Achromobacteriaceae	31	19	16	65
<i>Bacteroides</i> sp.	12	0	50	50
<i>Clostridium</i> sp.	12	100	0	0

One child vomited after 24 hours on the drug. Another child developed diarrhea after five days of therapy. One 11 year old child, with unexplained fever, developed icterus and fever after three weeks of therapy with increasing dosage from 1 to 2 Gm./day. The laboratory data suggested the possibility of acute hepatitis with clinical and laboratory clearance of the jaundice and reduction of hepatomegaly within two weeks after the drug was discontinued. The drug was well tolerated by the remainder of the patients.

#### SENSITIVITY OF ROUTINELY ISOLATED BACTERIA

While this clinical investigation was in progress, all bacteria isolated routinely were tested for sensitivity to oleandomycin by the disc method. The results are revealed in table II and indicate a high incidence of sensitive strains among the staphylococci, streptococci, pneumococci, and clostridia.

#### SUMMARY AND CONCLUSIONS

Sixty-two infants, children, and a few adults, with pneumonia, otitis media, cervical adenitis, and furunculosis, and other infections, were treated orally with triacetyloleandomycin, 40 mg./Kg./day in children and 2 Gm. daily for adults, with good results in 51, slow improvement in 6, 1 recurrence, and 5 failures to respond. The only failures encountered were in infants and children with chronic pulmonary disease and in one instance of leukemia, in which the underlying pathology precluded complete cure; only temporary improvement has been observed with antibiotics. Carriers of the 80/81 strain of *Staphylococcus aureus* involved in a nursery epidemic were cleared promptly of the offending agent. Furunculosis and infected family contacts were treated simultaneously and successfully. Toxic effects were very few and mild, except for one case of hepatitis that probably was coincidental and of the infectious variety. The sensitivity spectrum of 2963 strains of bacteria is reported.

#### ADDENDUM

Since the paper was written, we have had three recurrences of furuncles in patients who seemed successfully treated with triacetyloleandomycin. They were free for as long as two months but now again have boils; all have promptly improved with further triacetyloleandomycin therapy. We have observed icterus in another child. This girl of 9 had been in the hospital at the beginning of 1958 for incision of an abscess. She was re-admitted on August 4, 1958, with pleurisy and effusion. The effusion cleared on treatment with 2 Gm. of chloramphenicol daily for seven days. The chest was not tapped. On August 26 she was re-admitted because of fever and abdominal pains. She was found to have osteomyelitis of T-11 and T-12. On August 27, triacetyloleandomycin was started, 500 mg. every six hours. It was continued until September 20. On September 14, the mother noticed that the urine was orange and the stools very light and, on September 17, that the patient's eyes were yellow. Triacetyloleandomycin was discontinued on September 20. During hospitalization for pleurisy, liver function studies were made. Bilirubin, thymol turbidity, and cephalin flocculation were all negative; urine urobilinogen was 1/50 and bile, 0. Agglutinations for enteric pathogens were negative. On September 23 the sclerae were icteric; the liver was not tender nor enlarged; the spleen was not palpable; the bilirubin total, 1.4; alkaline phosphatase, 17 King-Armstrong units; thymol turbidity, 4.7; urea nitrogen, 18; erythrocyte sedimentation rate, 38; hematocrit, 39; urine clear without bile; urobilinogen, 1/8; and cephalin flocculation, 0, for 24 and 48 hours. The jaundice cleared rapidly, but there was still scleral staining on September 27, when the patient was discharged from the hospital. She had not been transfused or given human sera. The dosage of triacetyloleandomycin was 50 mg./Kg. It is believed that this was probably a toxic hepatitis due to triacetyloleandomycin, but, as in the previous instance, there were extenuating circumstances.

#### ACKNOWLEDGMENT

The triacetyloleandomycin used in this study was kindly supplied by Chas. Pfizer & Co.

# An Evaluation of Tetracycline-Oleandomycin in the Treatment of Epidemic Typhus

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It is well known that rickettsial infections, including typhus, are highly susceptible to broad-spectrum antibiotics. Results have been similar in epidemic and endemic typhus and in Brill's disease. The first reports were based on treatment with chloramphenicol. In a report of 5 cases of endemic typhus<sup>1</sup> response was dramatic to this antibiotic. In 8 patients treated with 2 to 3 Gm. of chloramphenicol daily,<sup>2</sup> fever disappeared in an average of 46 hours, with no recurrences. In 14 patients<sup>3</sup> treated with chloramphenicol at a dose of 50 to 75 mg./Kg. of body weight during 72 hours, fever disappeared in 30 to 120 hours, with 70 hours as an average; 6 recurrences were recorded, probably because the antibiotic was withdrawn early, although some authors believe this is a result of a lower activity, compared with that of the tetracyclines. There are also some reports of the effectiveness of the tetracyclines on the treatment of endemic typhus. Knight and co-workers<sup>4</sup> treated 60 endemic typhus patients with chlortetracycline, using different therapeutic schedules. The first group consisted of 18 cases, who received doses of 80 to 200 mg./Kg. of body weight daily during six days, with a disappearance of clinical symptoms and fever in approximately 38 hours; therapy was started on the eighth day of fever; the second group consisted of 24 cases who were administered the antibiotic at a dose of 50 to 75 mg./Kg. of body weight, and both fever and clinical symptoms disappeared in approximately 42 hours; therapy was begun on the sixth day of fever; a third group composed of 18 cases were given chlortetracycline at a dose of 50 to 72 mg./Kg. of body weight during one to two days; both fever and clinical symptoms disappeared in about 42 hours; therapy was started on the ninth day of infection; no recurrences were recorded in any case. Identical results have been reported with oxytetracycline. In 4 cases who were administered this antibiotic at a dose of 50 mg./Kg. of body weight daily during 1 to 6.5 days, fever was eliminated in approximately 70 hours, with limits of 48 to 132 hours.

Results have been very similar in the treatment of epidemic typhus with broad-spectrum antibiotics. Payne et al<sup>5</sup> treated 22 cases of epidemic typhus with chloramphenicol at a dose of 1 to 3.5 Gm. daily during one to three days, with dramatic responses. Fever disappeared in 24 to 56 hours, with an average of 34 hours; no recurrences or fatalities were recorded; these results are in contrast with a group of 50 patients who did not receive the antibiotic and of whom 14 (28 per cent) died. Pfeiffer<sup>6</sup> treated 6 cases of epidemic typhus in South Africa with chlortetracycline, eliminating fever in an average of 48 hours. Three patients of his series, who were given oxytetracycline at a dose of 75 mg./Kg. of body weight daily during three days were free of fever and clinical symptoms in 36 to 45 hours. Zapff-Grosse<sup>7</sup> treated 24 cases of epidemic typhus with oxytetracycline and recorded a normal temperature in 80 hours as an average, with no fatalities. Killough and Magill<sup>8</sup> treated 5 cases of epidemic typhus with oxytetracycline at a dose of 75 mg./Kg. of body weight daily during four days, which made fever disappear in three to five days, with no recurrences.

Three cases have been reported on Brill's disease treated with chlortetracycline.<sup>9</sup> Both fever and clinical symptoms disappeared in 14 to 48 hours. A case treated

\* The trade name of Chas. Pfizer & Co. for tetracycline-oleandomycin is Signemycin.

TABLE I  
Specific Titration

Child	Weil-Felix reaction	Rickettsiae agglutination reaction		Weil-Felix surface reaction, %
		<i>R. prowazekii</i>	<i>R. mooseri</i>	
C. C. J.	1:40	++	—	30
A. G. C.	1:5120	+++	+	95
V. F. J.	1:20	+	—	10
H. R. A.	1:1280	+++	—	80
P. R. V.	1:400	+	—	70
M. C. I.	1:1280	++++	—	90
C. C. J.	1:40	++	—	30
B. E. G.	1:1280	++++	—	95
D. L. R.	1:640	1:640	1:40	75
B. S. J.	—	—	—	30
L. G. B.	1:160	—	—	30
G. B. V.	1:80	1:10	—	50
H. M.	1:20	—	—	20
M. J. L.	1:1280	1:320	1:1	80
E. O. R.	1:40	—	—	20
E. E.	1:640	1:640	1:160	90

with chloramphenicol at a dose of 4 Gm. daily during 3.5 days was free of fever between the third and the fifth days.<sup>10</sup>

Ruiz Sánchez and co-workers<sup>11</sup> treated 6 cases of typhus with tetracycline hydrochloride. Medication was started between the sixth and the eleventh days of the infection. Doses ranged from 50 to 70 mg./Kg. of body weight daily during three to four days. Temperature became normal in 36 to 84 hours, with an average of 56 hours. Clinical symptoms disappeared in the same period. No complications or recurrences were recorded.

#### MATERIAL AND METHODS

During an epidemic of exanthematous typhus in several children's protection houses in Mexico City, which was confirmed by epidemiologic data and evident clini-

TABLE II  
Results of Treatment of 20 Cases of Epidemic Typhus\*

Case no.	Age, yr.	Days of evolution before treatment	Days of treatment	Dose, mg./Kg. body weight	Duration of fever, hr.	Days in hospital
1	11	15	1	50	—	3
2	11	16	1	50	—	5
3	9	9	5	50	76	6
4	12	6	5	50	72	7
5	8	8	5	50	48	12
6	14	6	5	50	78	6
7	12	18	1	50	—	10
8	9	7	5	50	60	10
9	10	10	5	50	48	9
10	11	7	5	50	72	4
11	12	7	5	50	36	7
12	13	5	5	50	84	12
13	13	6	7	50	108	8
14	13	3	5	50	72	8
15	7	12	2	50	—	4
16	12	7	4	50	36	8
17	10	12	2	50	144	14
18	7	5	5	50	66	6
19	7	15	5	50	72	10
20	7	6	5	50	67	5

\* All cases were treated with tetracycline-oleandomycin except cases 17, 18, 19, and 20. Case 17 was treated first with erythromycin and later with chloramphenicol. Cases 18 and 19 were treated with chloramphenicol and tetracycline alone respectively. Case number 20 was treated with chloramphenicol alone.

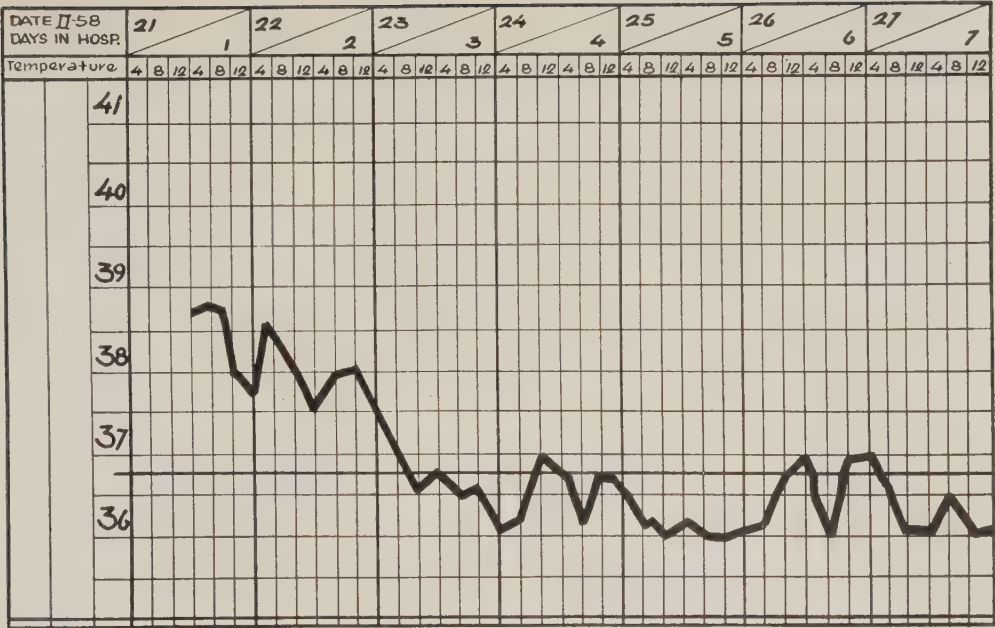


FIG. 1. The graph gives the thermic curve response to treatment in a 12 year old epidemic typhus patient. Temperature became normal in 36 hours.

cal symptoms associated with high agglutination rates in more than 50 per cent of 250 serum samples, we had the opportunity of treating cases who were admitted to the Children's Hospital.

A total of 20 cases of epidemic typhus was admitted, most of them with typical symptoms consisting of high fever ranging from 39 to 41 C. stupor, headache, photophobia, characteristic facial signs, conjunctival congestion, arthralgia, myalgia, classic exanthema in some patients and petechiae in others, distributed mainly in the chest and limbs. In all cases a positive Weil-Felix reaction was obtained. Agglutination was marked with *Rickettsia prowazekii*. In several cases antibodies were titrated, with the highest rate for *R. prowazekii* and a lower titer for *Rickettsia*

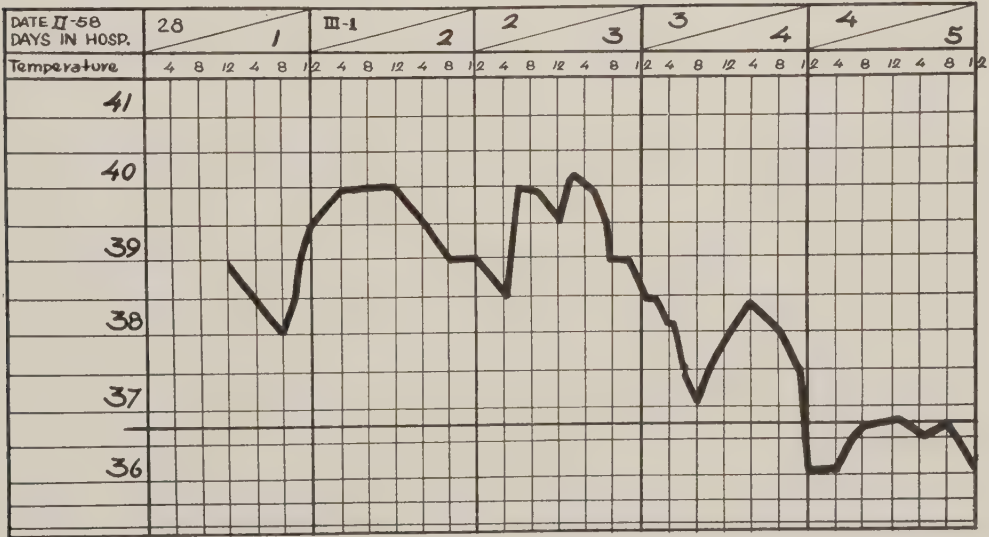


FIG. 2. Illustrated is the thermic response to treatment in a 13 year old patient. Fever disappeared in 84 hours.

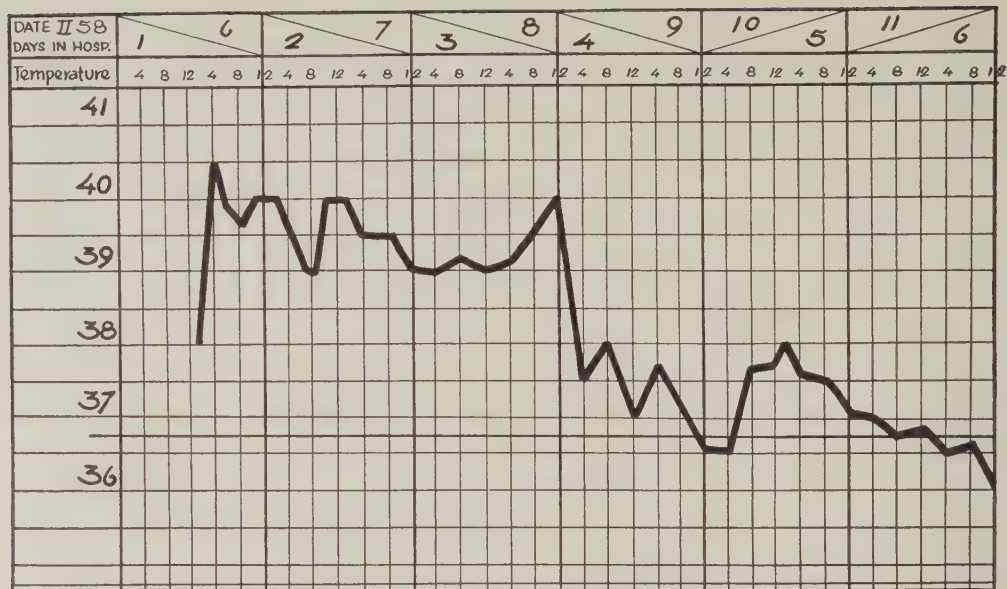


FIG. 3. The fever response to antibiotic steroid treatment in a 13 year old patient is shown. Temperature normalized in 108 hours.

mooseri. However, not all cases had a high antibody titer. Those patients admitted in the convalescence period presented the highest rates. *R. prowazekii* was cultured. The ages of patients ranged from 7 to 14 years. All were men. White cell counts were variable and ranged from 6000 to 10,000/cu. mm. leukocytes, but in some cases there were up to 20,000 to 30,000 white cells/cu. mm., with neutrophils as the predominating elements. Blood sedimentation was fast. Urine had a low amount of leukocytes, biliary pigments, albumin, or red blood cells. Electrocardiographs were normal in the few cases in which it was taken. Blood pressure varied between normal limits. Several patients had infectious complications before antibiotic therapy was started, including otitis, mastoiditis, pneumonia, bronchitis, and gangrene, that subsided soon after therapy was begun. A high percentage of the patients had very severe symptoms, particularly among the elder ones. Four children were admitted in a period of convalescence, with no fever (table I).

#### TREATMENT

As soon as the patients were admitted to the hospital, antibiotics were administered. Therapy was initiated between the third and the tenth days of the infection. Tetracycline-oleandomycin was given orally to 16 patients, at a dose of 50 mg./Kg. of body weight daily, at six hour intervals for five days. Four patients who were admitted in the convalescent period, with no fever, received these antibiotics for one or two days. One case was treated with tetracycline hydrochloride; a second patient was administered erythromycin initially, and then chloramphenicol; 2 more cases were treated with chloramphenicol (table II).

#### RESULTS

The response to tetracycline-oleandomycin can be considered dramatic, since all clinical symptoms disappeared in two to three days. Stupor, delirium, headache, and myalgia were eliminated in 48 hours. Exanthema and petechiae were present for a longer period. Fever was checked in 36 to 84 hours, with an average of 67

hours after therapy was started (figs. 1, 2). Only in one case, fever lasted 108 hours. The patient was additionally given steroids (fig. 3).

A case treated with erythromycin did not respond and the erythromycin was substituted for chloramphenicol. Clinical symptoms and fever were controlled in 144 hours (case 17). Two more cases treated with chloramphenicol and tetracycline, respectively, responded between 67 and 72 hours (cases 18 and 19). A fourth case was given chloramphenicol (case 20). This patient died probably of thrombosis as a result of arteritis of the femoral vessels plus gangrene. Complications in several patients, such as otitis, mastoiditis, pneumonia, and bronchitis, were controlled with tetracycline-oleandomycin. No recurrences were recorded.

#### DISCUSSION

Tetracycline-oleandomycin has been tested both in vivo and in vitro for its antibacterial action by several investigators.<sup>12-32</sup> According to some authors, these tests may show a synergistic activity between the two components. Our experience in the treatment of varied infections, including purulent meningitis, bacterial bronchopneumonia in children and peritonitis after visceral perforation shows favorable results, better than those obtained with tetracycline alone. This could possibly be explained through a supplementation of the antimicrobial action of each of the two components when they are associated. On the other hand, it is well known that superinfections are often a consequence of antibiotic therapy in hospitals, and oleandomycin may help to prevent this risk, specially when it is associated with tetracycline. This antibiotic association has proved a great value in bacterial bronchopneumonia in children younger than 2 years old where *Micrococcus pyogenes* var. *aureus* is the infectious agent in one third of cases.

In the treatment of a series of typhus cases we had the opportunity of comparing the results obtained with tetracycline-oleandomycin with those reported on the use of other broad-spectrum antibiotics. Our dosage schedule was generally lower than the ones used by other investigators with a broad-spectrum antibiotic alone, which are of the order of 50 to 200 mg./Kg. of body weight a day, compared with the 50 mg./Kg./day of tetracycline-oleandomycin reported in this series, which is equal to 33 mg. of tetracycline and 17 mg. of oleandomycin per Kg. of body weight. Responses were parallel to those obtained with other broad-spectrum antibiotics alone, at higher doses.

#### SUMMARY

A series of 16 typhus cases of a total of 20 patients admitted during an epidemic, and they were treated with a combination of tetracycline-oleandomycin. Therapy was started within the first 10 days of the infection.

The response can be considered dramatic. Clinical symptoms disappeared in two to three days, and fever was controlled in 36 to 84 hours, within an average of 67 hours. In one case only the fever lasted 108 hours.

Tetracycline-oleandomycin appears to be an effective therapeutic agent for typhus infections.

#### BIBLIOGRAPHY

1. KNIGHT, V.: Clinical evaluation of aureomycin, chloramphenicol, and terramycin. New York J. Med. 50:2173, 1950.
2. SMADEL, J. E.; LEÓN, A. P.; LEY, H. L., JR., AND VARELA, G.: Chloramphenicol (chloromycetin) in the treatment of patients with typhus fever. Proc. Soc. Exper. Biol. & Med. 68:12, 1958.
3. SMADEL, J. E.; BAILEY, C. A., AND DIECKS, F. H.: Chloramphenicol (chloromycetin) in the chemoprophylaxis of scrub typhus in treated volunteers and their prevention, Am. J. Hyg. 51:229, 1950.

4. KNIGHT, V.; RUIZ SÁNCHEZ, F.; RUIZ SÁNCHEZ, A., AND McDERMOTT, W.: Aureomycin in typhus and brucellosis, *Am. J. Med.* 6:407, 1949.
5. PAYNE, E. H.; SHARP, E. A., AND KNAUDT, J. A.: Treatment of epidemic typhus with chloromycetin, *Tr. Roy. Soc. Trop. Med. & Hyg.* 43:163, 1948.
6. PFEIFFER, D. H.: Louse and flea-borne typhus. Cerebro-spinal fluid findings and the response to aureomycin and chloromycetin, *South African M.J.* 24:111, 1950.
7. ZAPFF-GROSSE, R.: Terramycin in the treatment of exanthematous typhus (typhus fever). Presented at Conference on Terramycin, Lima, Perú. Dec. 6-7, 1951.
8. KILLOUGH, J. H., AND MAGILL, G. B.: Terramycin in epidemic typhus, amebic dysentery and typhoid, *J.A.M.A.* 147:1737, 1950.
9. BLUMBERG, N.; DOANE, J. C., AND WEISS, L. B.: Brill's disease: Report of three cases with aureomycin treatment in two, *New England J. Med.* 241:479, 1949.
10. MURRAY, E.; BAEHR, G.; SCHWARZMANN, G.; MANDELBAUM, R. A.; ROSENTHAL, J.; DOANE, J. C.; WEISS, L. B.; COHEN, S., AND SNYDER, J. C.: Brill's disease, *J.A.M.A.* 142:1059, 1950.
11. RUIZ SÁNCHEZ, F.; RUIZ SÁNCHEZ, A.; BECERRA, A.; NARANJO GRANDA, E.: Treatment of exanthematic typhus with tetracycline, *Antib. & Chemo.* 4:402, 1954.
12. MOLINELLI, E. A.; VERA BARROS, E.; ITHURRALDE, D.: Tetracycline-oleandomycin in human brucellosis therapy. Preliminary observations. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; p. 692.
13. LOUGHLIN, E. H., AND MULLIN, W. G.: Combined antibiotic therapy of tropical infections with oxytetracycline-oleandomycin and tetracycline-oleandomycin. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; p. 698.
14. MORADOR, J. L., AND SALDAÑA TATE, L.: Treatment of fifty-two cases of infections caused by coagulase-positive staphylococci with a combination of oleandomycin and tetracycline. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; p. 702.
15. ENGLISH, A. R.; McBRIDE, T.; VAN HALSEMA, G., AND CARLOZZI, M.: Biologic studies of PA 775, a combination of tetracycline and oleandomycin with synergistic activity, *Antib. & Chemo.* 6:511, 1956.
16. FINLAND, M., AND HAIGHT, T.: Antibiotic resistance of pathogenic staphylococcus, *A.M.A. Arch. Int. Med.* 91:143, 1953.
17. CIMMINO, A.; BONI, A., AND ORSI, N.: Antibiotic activity of oleandomycin-tetracycline combination. In vitro study on 332 strains of *Micrococcus pyogenes* var. *aureus*, clinically isolated. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; p. 708.
18. JAWETZ, G., AND GUNNISON, J. B.: Antibiotic synergism and antagonism and assessment of the problem, *Pharmacol. Rev.* 5:175, 1953.
19. CARTER, C. H., AND MALEY, M. C.: Application of tetracycline-oleandomycin in clinical practice. A preliminary report. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957; p. 51.
20. WINTON, S. S., AND CHESROW, E. J.: A clinical study of combined chemotherapy: tetracycline and oleandomycin. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957; p. 55.
21. LOUGHLIN, E. H., AND MULLIN, W. G.: Combined antibiotic therapy of tropical infections with PA 765 PA 775, a preliminary report. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957; p. 63.
22. LA CAILLE, R. A., AND PRIGOT, A.: Combination of oleandomycin with oxytetracycline and tetracycline in the treatment of soft-tissue infections. A preliminary report. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957; p. 67.
23. ELLIOTT, H. J., AND HALL, W. H.: Activity of oleandomycin salts, mixtures, and combinations against staphylococci. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; p. 716.
24. JONES, W. G., AND FINLAND, M.: Antibiotics combinations, *New England J. Med.* 256:115, 1957.
25. LEVITT, R. O., AND HUBBLE, R. H.: Antibiotics mixtures: In vitro effect of tetracycline-oleandomycin mixture (PA 775) on staphylococci, *New England J. Med.* 257:180, 1957.
26. HALL, W. H., AND ELLIOTT, H. J.: The effect of antibiotic combinations upon staphylococci, with special reference to oleandomycin, *J. Lab. & Clin. Med.* 50:822, 1957.
27. ROSS, S.; ZAREMBA, E. A., AND PUIG, J. R.: Some comments on the lack of synergism of tetracycline-oleandomycin mixture. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; p. 723.
28. FOULKE, C. W., AND ROMANSKY, M. J.: An in vitro study of oleandomycin-tetracycline mixture against 103 strains of hemolytic *Staphylococcus aureus*. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; p. 732.
29. ANON.: The clinical use of antibiotics in combination (Editorial), *A.M.A. Arch. Int. Med.* 99:536, 1957.
30. ANON.: Oleandomycin-tetracycline mixture (Editorial), *New England J. Med.* 257:289, 1957; 255:1057, 1957.
31. WELCH, H.; WRIGHT, W. W.; REEDY, R. J., AND WINTERMERE, D.: Antibiotic combinations: In vitro effects on selected groups of bacteria. I. Combinations of oleandomycin with sixteen other antibiotics. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; p. 738.
32. OSWALD, E. J., AND WELCH, H.: Antibiotics combinations. III. In vitro effects of oleandomycin-tetracycline on two hundred and two cultures of *Micrococcus pyogenes* var. *aureus*. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; p. 751.

# **Tetracycline-Oleandomycin in the Treatment of Certain Infections Encountered in the Tropics**

## **With Particular Reference to the Use of Combined Antibiotic Therapy of Amebiasis, Yaws, Lymphogranuloma Venereum, Tropical Ulcer, and Acute Gonococcal Urethritis**

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Continuing the studies on the treatment of certain tropical infections with the antibiotic combinations of oxytetracycline and oleandomycin and of tetracycline and oleandomycin (Signemycin\*) that we started early in 1956, emphasis was placed during the past year on further clinical investigations with the latter combination. Although some of the data included in this article was reported in earlier publications,<sup>1-3</sup> we believe that we have accumulated enough new cases treated with the combination, particularly chronic intestinal amebiasis as well as previously unreported cases of acute gonorrhea, to warrant their presentation.

### DOSAGE

The dosage of this antibiotic combination of tetracycline and oleandomycin, as we have stated in two previous papers, was semi-empirical, and instead of being based on weight, it was based on age. Thus, in chronic intestinal amebiasis and in yaws, the following doses were given once daily: to children less than 5 years old, three 250 mg. capsules (each containing 167 mg. of tetracycline and 83 mg. of oleandomycin) or their equivalent in an oral suspension; to those between 5 and 10 years old, four capsules; to those more than 10 years old and to adults, six capsules. The duration of dosage necessarily varied for each disease. Thus, in chronic intestinal amebiasis this antibiotic combination was given for 10 consecutive days, while in yaws, it was given for five consecutive days.

In early cases of lymphogranuloma venereum, two 250 mg. capsules of tetracycline-oleandomycin were given three times daily for periods of four days, while in later cases of the inguinal, pelvic, and anorectal syndromes (as an adjunctive prior to surgery), this dosage was administered for 14 consecutive days.

In Haiti, where tropical phagadenic ulcer occurs in regions where yaws has been endemic, the systemic dosage of this combination of tetracycline and oleandomycin was the same as that used for treating yaws.

This antibiotic combination was applied topically to large bacterially contaminated ulcerous lesions of early and late yaws, and generally to tropical phagadenic ulcers until they were closed over.

In acute gonococcal urethritis, a single dose of six 250 mg. capsules of tetracycline-oleandomycin was given.

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\* The trade name of Chas. Pfizer & Co. for tetracycline-oleandomycin is Signemycin.

Eighty-six patients with chronic intestinal amebiasis, ages ranging from 6 to 67 years old, were treated with this combination of tetracycline and oleandomycin. The symptoms of 59 of these patients could be ascribed reasonably to infection with *Endamoeba histolytica*. The remainder either had no symptoms referable to the infection, or, because of coexisting multiple parasitisms, their symptoms could not be appraised.

The diagnosis of intestinal amebiasis was established by finding the cysts of *E. histolytica* in fecal smears and in SAEX concentrations.<sup>4</sup> The results of treatment were evaluated lately by similarly examining the feces for cysts of this protozoan on the last day of therapy and then at weekly intervals during a minimum of six weeks post-treatment. As we have stated before, the post-treatment period of observation was limited to six weeks, so as to reduce the possibility of confusing reinfection with relapse, should *E. histolytica* reappear in the feces during or after that time.

All of these patients became negative for *E. histolytica* by the time of the first (tenth day) treatment examination. Eighty-two patients remained negative during the six weeks post-treatment period. Four patients, 2 of whom had cysts of *E. histolytica* in the feces at four weeks post-treatment, and 2 of whom had cysts of this protozoan in the feces at six weeks, were considered to have relapsed.

#### YAWS

One hundred and seven patients with yaws, ages ranging from 2 to 70 years were treated with the combination. Eighty-eight patients had early yaws, including primary lesions and such secondary lesions as frambesiomias and ulcerous plantar lesions. Nineteen patients had late yaws lesions, including late plantar lesions, osteoperiostitis, and gummas. The patients with early yaws frequently had the characteristic systemic symptoms of infection with *Treponema pertenue* as well as enlarged inguinal and submental lymph nodes.

Primary lesions uniformly were dry by the second or third day of treatment and, generally, by the fourth or fifth day were covered by crusts. Smaller primary lesions regularly were healed by the eighth to tenth days, while larger ulcerous primary lesions usually required two to three weeks for complete healing to take place.

Frambesiomias of the face, limbs, and trunk regularly were skinned over within five to six days after starting treatment, and by the seventh to tenth days, their former sites could be identified only by altered pigmentation, slight erythema, or wrinkling of previously affected skin. Although perianal and genital frambesiomias were somewhat slower to recede, at two weeks post-treatment, only slightly thickened and wrinkled skin or altered pigmentation marked their sites.

Plantar lesions were rendered painless within 24 to 48 hours, and by the third day the patients were able to walk gently on the affected parts of the soles. Usually, from the fifth day on they had useful lower extremities.

The systemic manifestations of early yaws disappeared rapidly during therapy with tetracycline-oleandomycin.

One patient, who was treated for early yaws, relapsed after two months.

The results of treatment of late yaws were just as remarkable as some of those that we described with treatment by oxytetracycline. In 3 cases of osteoperiostitis, it was necessary to repeat the course of therapy with the combination.

Ninety-five patients with lymphogranuloma venereum were treated with tetracycline-oleandomycin. Seventy-nine patients had the inguinal syndrome (buboes), 5 had the pelvic syndrome, and 11 had the anorectal syndrome. One of these had vulvar ulceration, and 4 of the latter had progressed to fibrotic strictures. The clinical diagnosis of lymphogranuloma venereum was confirmed by a positive skin test (Frei) and/or positive complement-fixation test.

Regression of the lymphadenitis (buboes) was observed usually by the fourth or fifth day and generally was complete within two to three weeks. Aspiration or incision of buboes was not required. In 23 cases the dosage of the antibiotic combination was repeated after one week, since it was thought that regression of the lymphadenitis was comparatively slow. The inflammatory stages of the rectal syndrome regressed satisfactorily with the combination. When used as an adjunctive, the combination permitted considerable shortening of the presurgical period of antibiotic treatment. The results of surgery also were usually better with this regimen using the antibiotic combination than with those using only a single antibiotic. There were no relapses during periods of no less than three months' post-treatment.

#### TROPICAL ULCER

Seventy-eight patients with chronic phagadenic or tropical ulcers, ranging in age from 25 to 68 years old, were treated with this combination of tetracycline and oleandomycin.

Since these ulcers had started from several months to as many as 18 years earlier, rapid healing was not expected. However, as we have reported before, the ulcers regularly became drier during the first two or three days of combined systemic and topical therapy with this antibiotic combination, and within one week usually were reduced in size by one-quarter to one-third. Ulcers less than 4 to 5 mm. deep and 5 to 7 cm. across usually were completely healed within two to three weeks. Larger ulcers with exposed muscle and tendon sheaths were slower in healing, but even these frequently were reduced in size by one-quarter during the third to fourth weeks. Ulcers with heavy bacterial contamination and foul exudate became cleaner and drier by the third day of treatment. Edema of contiguous tissues, even when of long standing, was reduced within 48 hours. Patients, whose ulcers had not healed completely after three weeks of topical therapy, were permitted to return home to continue topical applications of this antibiotic combination.

#### ACUTE GONOCOCCAL URETHRITIS

Fifty-four men were treated with tetracycline-oleandomycin.

The diagnosis of acute gonococcal urethritis was determined by a history of exposure, local physical examination, and finding gonococci in Gram-stained spreads of urethral exudate.

The patients, after receiving their dose of the combination were told to return for re-examination on the second or third day post-treatment and again on the seventh and fourteenth days post-treatment. At these times, spreads and cultures of urethral discharge, if any, were made and examined for gonococci. When bacteriological examinations were negative at the first two post-treatment visits, and the clinical manifestations of urethritis had disappeared, cure was thought to have been accomplished.

Using these criteria, 53 of these 54 patients were considered to have been cured of their infection.

#### TOXIC REACTIONS

This antibiotic combination of tetracycline and oleandomycin, when administered systemically in these dosages to patients previously described, caused no adverse reactions. Except for mild stinging or burning, there were no adverse effects provoked by the topical application of the tetracycline-oleandomycin combination. Local allergic sensitization of the skin or tissues did not occur as a result of such topical application.

#### BIBLIOGRAPHY

1. LOUGHLIN, E. H., AND MULLIN, W. G.: The antibiotics in tropical medicine, *Internat. Rec. Med.* 169:489-505, 1956.
2. LOUGHLIN, E. H., AND MULLIN, W. G.: Combined antibiotic therapy of tropical infections with PA 765 and PA 775: a preliminary report. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 63.
3. LOUGHLIN, E. H., AND MULLIN, W. G.: Combined antibiotic therapy of tropical infections with oxytetracycline-oleandomycin and tetracycline-oleandomycin. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 698.
4. LOUGHLIN, E. H., AND SPITZ, S. H.: Diagnosis of helminthiasis, *J.A.M.A.* 139:997-1000, 1949.

# The Use of Tetracycline-Oleandomycin in Buccal Surgery

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There has been a continual increase in the use of antibiotic combinations due to their synergistic activity, i.e., joint action of constituents of the antibiotics, which strengthens their reciprocal potency or brings about a different effect from those produced by each antibiotic separately.

This synergistic quality has great value, especially in treating organisms resistant to individual antibiotics.

With the object of finding an antibiotic suitable for treatment of most buccal infections, we chose to study an antibiotic combination with synergistic action—tetracycline-oleandomycin (Sigmamycin\*).

## EXPERIMENTAL CLINICAL STUDIES

In order to establish length of treatment, best method of administration of the drug, and dosage, we have set up two main study groups of patients, according to whether the antibiotic therapy was given preoperatively or postoperatively.

Patients in the first group are subdivided according to whether they have septic or aseptic processes. Those in the second group are subdivided according to whether the infectious process is mild, moderate, or severe. In the preoperative group, we consider to have aseptic processes those patients whose need for surgery has no relation whatsoever—apparent or not—to infection. We consider to have septic surgical processes those patients with chronic infectious conditions such as abscess, granuloma, infected cysts, teeth retained with recurrent pericoronitis, chronic sinusitis, torpid osteomyelitis. We do not include, of course, in this group patients with acute infections dominating the clinical picture, because in these patients, from the standpoint of antibiotic therapy, the pre- and postoperative treatment are considered as one.

In the postoperative group, consider mild infections those with a limited clinical picture and with no spreading tendency, local or general, such as: alveolitis, simple pericoronitis, localized phlegmon of the superior maxilla, early Vincent's stomatitis. As moderate infections, we include phlegmonous processes in various sites with spreading tendency but with no general effect on the organism, for example: phlegmons in the base of mouth and of the xygomatic fossa, sequestering osteomyelitis. Finally, we consider as severe processes those just mentioned but having a general effect (fever, intoxication).

Dosage in each case was systematized according to the disease in order to facilitate the interpretation and control of results obtained. We administered in the preoperative period 1 capsule of 250 mg. every six hours for aseptic processes and 2 capsules in the same period for five days in septic processes, i.e., two days before operation, day of operation, and two days after, providing, of course, there was no complication.

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\* The trade name of Chas. Pfizer & Co. for a tetracycline-oleandomycin combination is Sigmamycin.

TABLE I  
*Dosage of Tetracycline-Oleandomycin*

Group		Dosage in 24 hours
Preoperative	Aseptic	4 capsules (1 Gm.) for 5 days
	Septic	8 capsules (2 Gm.) for 5 days
Settled infectious process	Mild	6 capsules (1.5 Gm.) until 48 hours after remission of process
	Moderate	12 capsules (3 Gm.) until 48 hours after remission of process
	Severe	24 capsules (6 Gm.) until 48 hours after remission of process

In mild acute processes we administered 1 capsule every four hours, maintaining medication for 24 or 48 hours after regression of the infection. In moderate cases we gave 2 capsules every four hours, maintaining medication as just described. In patients with severe infections we employed 6 Gm. daily as maximum dosage, 6 capsules every six hours, to obtain remission of the infection, and as in any other case, we also used evacuating surgery when necessary. In children less than 12, we advise half the dosage used for adults in every settled infectious process.

Table I shows optimum dosage in each case; table II shows results obtained with tetracycline-oleandomycin; and table III shows number of patients in each group.

#### RESULTS AND CONCLUSIONS

There were no sensitive reactions to the drug, and resistant strains were not apparent. Diarrhea with intestinal lability, indicating a predisposed field, was noted in only a few cases (1.5 per cent).

In every case in which we administered the antibiotic as a preoperative medication, its protecting action has been extremely satisfactory, and there has been no postoperative evidence of superinfection due to dissemination of pre-existent infectious foci.

Mild infections began to heal within 48 to 72 hours; generally, surgical intervention was not necessary. In moderate infectious processes, invasion is quickly stopped, permitting surgical intervention and also limiting the use of drains, which always

TABLE II  
*Results Obtained with Tetracycline-Oleandomycin*

Group		No. cases	Results		
			Excellent	Good	Poor
Preoperative	Aseptic	26	26	—	—
	Septic	11	11	—	—
Acute infectious processes	Mild	20	20	—	—
	Moderate	24	20	3	1
	Severe	16	13	3	—
Total		97	90	6	1

TABLE III  
*Disease Conditions*

Diagnosis	No. patients
Teeth, retained	19
Tumors, noninfected	9
Tumors, infected	1
Cysts of dental origin	9
Cysts of dental origin, infected	4
Surgery (prosthetic purposes)	4
Prognathism	1
Salivary lithiasis	1
Maxillary sinusitis	3
Periapical complications	3
Alveolitis	5
Gingivitis	4
Stomatitis	2
Periodontitis	3
Osteomyelitis	7
Phlegmons	19
Adenopathy	2
Fracture	1

causes visible undesirable esthetic sequelae because of the region in which the oral surgeon is working. In severe infectious processes, with fever, intoxication, tetracycline-oleandomycin was always a valuable aid in surgery, for after drainage, there was almost always remission of general symptoms within 48 hours; sometimes the patient was afebrile within the first 24 hours.

In some patients with ganglionic complication, when surgical drainage left undesirable esthetic sequelae on the face, it was possible to recede the fluctuating process with only antibiotic therapy and cold applications on the affected ganglion.

# Further Clinical Studies with a Combination of Tetracycline and Oleandomycin in the Treatment of Various Infections

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The antibiotic activity of the association of tetracycline and oleandomycin\* has been studied by several investigators, both in the laboratory and in the treatment of different infectious conditions.<sup>1-5</sup> Previously we reported tests of the in vitro antimicrobial action of this association on staphylococcal strains susceptible or resistant to other of the commonly used antibiotics. Our experiences with this antibiotic combination have been satisfactory.<sup>5,6</sup>

The present paper deals with the treatment of 170 cases of various infections treated with these drugs.

## MATERIALS AND METHODS

Patients were selected among those who had a clearly identified infectious condition and divided into a first group of 151 children of both sexes, with ages ranging from a few weeks to 7 years, and a second group of 19 adult women, 22 to 30 years of age. Diagnosis was established in all cases on the basis of clinical symptoms and laboratory tests. The need and the convenience of receiving the association of tetracycline and oleandomycin, in view of the severity of the clinical picture, or either the fact that the infectious case had shown a clinical resistance to other antibiotics previously administered were taken as a basis for the selection of those patients who were treated with the antibiotic preparation. However, the great majority of patients had not received any medication previous to therapy with the tetracycline and oleandomycin combination. Isolation and cultures of the etiologic microorganisms were made in all cases. Susceptibility tests with the preparation were performed in the laboratory for almost every case before therapy was started. All organisms tested showed an adequate in vitro susceptibility to the antibiotics even in those cases in which clinical resistance had been observed to other antibacterial agents. The tube dilution method was used in all laboratory tests. A mixed group of symptoms and complications were present practically in all cases. A classification of cases, according to diagnosis and total number, is shown in table I.

Enteric infectious diarrhea, either alone or in association with other infections was the predominating condition in the infantile group, with 101 cases. Intrapartum sepsis was the most frequently found infection in the adult women group. Bacteriological isolates showed a high percentage of *Escherichia coli*, *Proteus*, and *Shigella* in the enteric diarrheal patients. *Streptococcus* and *Staphylococcus* appeared to be the main etiological microorganisms in the gynecological cases. Table II shows the frequency of appearance of the different organisms in each separate group of infections.

\* The trade name of Chas. Pfizer & Co. for tetracycline-oleandomycin is Signemycin.

TABLE I

Classification of the Various Infections According to Diagnosis and the Total Number of Cases

Diagnosis	Number of cases
<i>Infantile Patients</i>	
Bronchitis	5
Bronchitis complicating measles	3
Bronchitis complicating pharyngitis	1
Bronchitis complicating rhinopharyngitis	1
Bronchopneumonia	3
Bronchopneumonia complicating measles and postinfectious encephalitis	1
Bronchopneumonia complicating pertussis	2
Enteric infectious diarrhea	91
Enteric infectious diarrhea and bronchitis	4
Enteric infectious diarrhea and pharyngitis	2
Enteric infectious diarrhea and tonsillitis	1
Enteric infectious diarrhea complicating varicella	1
Enteric infectious diarrhea complicating varicella and laryngotracheobronchitis	2
Glomerulonephritis	1
Impetigo	2
Laryngopharyngitis	5
Laryngotracheitis	2
Laryngotracheitis complicating measles	1
Laryngotracheobronchitis	1
Laryngotracheobronchitis complicating varicella	1
Lobar pneumonia	1
Pertussis	1
Pertussis and bronchitis	2
Rheumatic fever	3
Scarlet fever	2
Suppurative otitis media and bronchitis	2
Suppurative otitis media and tonsillitis	1
Tonsillitis	7
Tonsillitis and pharyngitis	1
Typhoid fever	1
<i>Adults (Female Patients)</i>	
Endometritis	5
Endometritis and pelvic peritonitis	1
Intrapartum sepsis	13
	170

Symptomatology in cases of infection with *Salmonella typhi* was confined mainly to the digestive organs. Thus, these cases were considered among the digestive tract infections, in accordance to the classic concepts. *Micrococcus pyogenes* var. *aureus* was found to be the pathogenic organism of the highest incidence in both upper and lower respiratory tract infections, and in ear and skin conditions. In 2 cases of enteric infectious diarrhea, *M. pyogenes* var. *aureus* was also the causative organism. *E. coli* 0127 was isolated from a case of bronchopneumonia.

#### TREATMENT

All 170 patients were treated with a combination of tetracycline and oleandomycin, available in 250 mg. gelatin capsules, syrup, and intravenous preparations. The intravenous route was used in the most severe cases, and the oral route was chosen for the treatment of the rest of the patients, with a preference for syrup among the younger children. All infantile patients were administered a dosage schedule of 20 to 40 mg. of the antibiotic per Kg. of body weight orally every 24 hours, in equally divided doses at regular 6 hour intervals. Adult patients received

TABLE II  
Groups of Infections, Causative Microorganisms and Number of Isolates,  
by Order of Importance

Groups of infections	Microorganisms	Number of isolates
Upper respiratory tract	<i>M. pyogenes</i> var. <i>aureus</i>	6
	Alpha-hemolytic <i>Streptococcus</i>	5
	<i>Hemophilus influenzae</i>	4
	Beta-hemolytic <i>Streptococcus</i>	3
	<i>Diplococcus pneumoniae</i>	1
Lower respiratory tract	<i>M. pyogenes</i> var. <i>aureus</i>	8
	Alpha-hemolytic <i>Streptococcus</i>	7
	<i>Hemophilus influenzae</i>	6
	<i>Diplococcus pneumoniae</i>	4
	<i>Hemophilus pertussis</i>	4
	Beta-hemolytic <i>Streptococcus</i>	3
	<i>E. coli</i> 0127	1
Digestive tract	<i>E. coli</i> 0127	15
	<i>E. coli</i> 0111	14
	<i>E. coli</i> 0119	14
	<i>Shigella flexnerii</i>	12
	<i>Proteus vulgaris</i>	9
	<i>Proteus mirabilis</i>	8
	<i>E. coli</i> 055	7
	<i>S. typhi</i>	6
	<i>Proteus morganii</i>	6
	<i>Proteus rettgeri</i>	4
	<i>Shigella sonnei</i>	4
	<i>Shigella dysenteriae</i>	4
	Nonpathogenic coliformis	3
	<i>M. pyogenes</i> var. <i>aureus</i>	2
	<i>Shigella boydii</i>	2
	Paracolon bacilli	1
	<i>Enterobius vermicularis</i>	1
Genitourinary system	<i>M. pyogenes</i> var. <i>aureus</i>	17
	Alpha-hemolytic <i>Streptococcus</i>	14
	Beta-hemolytic <i>Streptococcus</i>	11
	<i>Proteus vulgaris</i>	6
	<i>Proteus mirabilis</i>	6
	<i>Trichomonas vaginalis</i>	5
Ear	<i>M. pyogenes</i> var. <i>aureus</i>	2
	Beta-hemolytic <i>Streptococcus</i>	1
	<i>M. pyogenes</i> var. <i>albus</i>	1
Skin	<i>M. pyogenes</i> var. <i>aureus</i>	1
	<i>M. pyogenes</i> var. <i>albus</i>	1
	Beta-hemolytic <i>Streptococcus</i>	1
Systemic infections	Alpha-hemolytic <i>Streptococcus</i>	2
	Beta-hemolytic <i>Streptococcus</i>	2

an initial oral dose of 500 mg., followed by 250 mg. every four or six hours. Variations in the oral doses were adjusted to the severity of symptoms. Whenever the intravenous route was used, adults were administered 1 to 2 Gm. every 24 hours in two to three divided doses. Infantile patients were administered 15 to 25 mg./Kg. of body weight every 24 hours, in equal doses divided at 6 to 8 hour intervals. Results were termed excellent when all clinical symptoms were controlled in 24 to 60 hours; good when symptomatology was eliminated in 60 to 72 hours; fair if symptoms were absent after 72 hours to 6 days; poor when more than six days were needed to control symptoms, and negative when there was no response to treatment and either it had to be suspended or the patient died. In no case was there a need to abandon treatment with the use of tetracycline and oleandomycin, and, of the 170 cases treated, only 1 had a negative response, in the enteric in-

fectious diarrheal group. This patient, a 3 year old boy, was admitted with a severe condition of water and electrolyte imbalance. Although adequate therapy was started immediately after admission, the patient died within four hours. Table III shows the results of therapy.

Interpretation of figures and results has to be made taking into consideration each different group of patients. Within certain limits, the time from the onset of symptoms to the start of therapy is markedly variable from one group to another, as it was also in each of the groups. No chronic or emergency cases were included in this series; the shortest time was eight hours before treatment, and the longest one was 12 days. Also, the shortest duration of therapy was 36 hours, and the longest was 10 days. However, the types of results are not directly given by the duration of therapy, since in many patients, whose results were considered excellent, treatment was continued for longer periods than the ones used for the classification of results, even after all symptoms were controlled. This is also valid for most of the other cases. Each group of infections consisted of a different and variable number of cases, and this accounts for the establishment of separate percentages of results for each group. This explains the fact that in a large group of patients, such as the enteric diarrheal with a total of 101 cases, a negative result means 1.01 per cent, while a single case of poor results mean 5 per cent in a much smaller group, like that of genitourinary infections, with 20 patients. Condensation of figures and results in this sense was performed with practical intentions.

The combination of tetracycline and oleandomycin was administered since the start of therapy. Additional therapeutic steps were taken whenever they were considered necessary and adequate. Parenteral administration of fluids and electrolytes, special diets, analgesics or antispasmodics, oxygen or a humid atmosphere, whole blood transfusions, and plasma were included.

Tolerance to the antibiotic was excellent in all cases, and only 1 patient had mild gastrointestinal symptoms, attributable to the drug. Clinical laboratory ex-

TABLE III  
*Case Grouping and Responses to Treatment with the Combination of  
Tetracycline and Oleandomycin*

Infectious group	Days of evolution before treatment	Duration of therapy	Responses, %
Upper respiratory tract	12 hours to 4 days	36 hours to 5 days	Excellent: 50 Good: 50
Lower respiratory tract	8 hours to 6 days	48 hours to 5 days	Excellent: 38 Good: 40 Fair: 22
Digestive tract	12 hours to 5 days	48 hours to 8 days	Excellent: 81.18 Good: 8.91 Fair: 4.95 Poor: 3.96 Negative: .99
Genitourinary	24 hours to 10 days	3 to 8 days	Excellent: 80 Good: 10 Fair: 5 Poor: 5
Ear	3 to 12 days	72 hours to 4 days	Good: 66.7 Fair: 33.3
Skin	4 days	48 hours	Excellent: 100
Systemic	3 to 12 days	60 hours to 10 days	Good: 50 Poor: 50

aminations did not show any changes that could be considered as a result of antibiotic therapy. Clinical resistance to the preparation did not develop in any case, and the only fatality in this series was considered as a result of the deep fluid and electrolyte imbalance present.

#### DISCUSSION

The results obtained from this study indicate that the association of tetracycline and oleandomycin is effective in the treatment of infections of a varied etiology, including those that show clinical resistance to the use of other antibacterial agents. The association has apparently a higher antimicrobial activity than that of either of its components alone, since a large group of infections caused by organisms usually difficult to deal with tetracycline, oleandomycin, or other antibiotics was controlled with the preparation. A mixed flora was generally present in the largest infectious group, that affecting the gastrointestinal system, but a clear predominance of strains of *E. coli* and *Proteus*, followed by *Shigella*, was noted in the laboratory isolates. *Proteus* specially, among these organisms, shows natural or acquired resistance to antibiotic therapy. However, all the infectious cases in which this species was present were controlled with the antibiotic treatment. Mixed bacterial species were also present in almost all of the rest of cases in this series, but since there was an obvious predominance of some symptoms, clinical case classifications were made in accordance with the main symptomatology. Development of bacterial resistance through adaptative mutations is apparently becoming a major problem of antimicrobial therapy, particularly among hospital populations, where contaminations by other patients or by carriers, including healthy personnel, are frequent. The great majority of patients in the present series had not been treated with other antibiotics before admission, but it is known that resistant infections can develop even in patients who are receiving an antibiotic for the first time. The association of tetracycline and oleandomycin appeared to prevent bacterial resistance in all cases, including those where antibiotics had been used before, with negative results in some patients.

#### SUMMARY

1. An association of tetracycline and oleandomycin was used in the treatment of 170 different infectious conditions, of a varied microbial etiology.
2. This antibiotic association was administered from the start of therapy, both in cases that had become resistant to other antibiotics and in those receiving antimicrobial therapy for the first time.
3. All the infectious conditions, caused in practically every case by a mixed flora, were effectively controlled by the therapeutic preparation, with the exception of one fatality, which was attributed to a very severe fluid and electrolyte imbalance that could not be corrected on time.
4. Used from the start of treatment, the tetracycline and oleandomycin combination confirmed clinically its broad-spectrum antimicrobial action and prevented bacterial resistance in all cases.

#### ACKNOWLEDGMENT

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## BIBLIOGRAPHY

1. ENGLISH, A. R.; MCBRIDE, T.; VAN HALSEMA, G., AND CARLOZZI, M.: Biologic studies on PA 775, a combination of tetracycline and oleandomycin with synergistic activity, *Antib. & Chemo.* 6:511, 1956.
2. WINTON, S. S., AND CHESROW, E.: A clinical study of combined chemotherapy: tetracycline and oleandomycin. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 55.
3. LA CAILLE, R. A., AND PRIGOT, A.: Combinations of oxytetracycline and tetracycline with oleandomycin in the treatment of soft-tissue infections: A preliminary report. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 67.
4. MOREY, G.: Infections in nursing babies due to *pseudomonas aeruginosa* alone or associated with other organisms, *Rev. del Hosp. del Niño*, 72:3, 1957.
5. HAGEN, H., AND SCHEFFLER, H.: Preliminary clinical experience with oleandomycin and the double-spectrum antibiotic sigmamycin, *Die Medizinische*, 14:477, 1957.
6. REBOLLEDO LARA, M., AND WARMAN, A.: Antibiotic susceptibility of gram-positive cocci isolated in México City hospitals. Ed. University of México School of Medicine, México, D.F., 1957.
7. HEREDIA DIAZ, J.; SAAVEDRA, G.; ROBLES, M. A., AND LOZA, D.: Tetracycline-oleandomycin (Sigmamycin) in the treatment of puerperic infections, *Medicina* 38:308, 1958.

# The Combination of Tetracycline-Oleandomycin in Pleuropulmonary Surgery

## Preliminary Report

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The practice of thoracic surgery requires the use of antibiotics in all its stages. In pleuropulmonary operations we deal with the infected bronchial tree, and the septic medium is even more important when surgical treatment is due to an infectious lesion. We have used all known antibiotics as we have had the opportunity to try them. Gradually, we favored the broad-spectrum antibiotics; we noticed, as did others, a progressive increase of resistant strains to penicillin. We tried oxytetracycline and chloramphenicol with satisfactory results; however, tolerance to them was not always complete and in some cases we observed cutaneous reactions and gastrointestinal disturbances.

In view of the antibiotic possibilities of synergism and potentiation, we decided to try an antibiotic combination in search of better tolerance and action.

We chose a combination of two thirds tetracycline and one third oleandomycin\* (167 and 83 mg. in a dose of 250 mg.).

We used this antibiotic in 55 patients: in 40, as a preventive medium before or after operation, and in 15, to treat infectious lesions or severe postoperative complications in which infection was an important factor.

### PREVENTIVE USE

We prescribed tetracycline-oleandomycin in 20 cases during the preoperative period. The dose was 1 Gm. daily (250 mg. every six hours) for two to five days immediately before operation. We had only one cutaneous reaction (5 per cent); and we did not record any cases of diarrhea or other gastrointestinal disturbances.

Another group of 20 patients received tetracycline-oleandomycin during the immediate postoperative period (table I). The dosage was 1 to 2 Gm. daily, given over a period of from 7 to 14 days.

Tolerance was complete. There were no cutaneous, mucous, or gastrointestinal reactions. In 18 patients, operation was done to treat lesions in which the infectious factor was not very important; there were no complications. In 2 patients operation was indicated to treat chronic pleural empyemas of six months' and of three years' duration, respectively. The first case was secondary to a severe pneumonitis and the second, to an intrapleural rupture of an hydatid cyst of the right lung. Previously, two pleural drainages were done in the last case. Both patients were decorticated, the empyematic sac being completely extirpated. In the prolonged and difficult operations it was impossible to avoid contamination of the wound; however, both healed without complications. In the second case, we noticed small serous secretions around one of the skin sutures, in which *Escherichia coli* was isolated; the organism was partly sensitive to tetracycline-oleandomycin; the wound healed once the stitch was taken out.

\* The trade name of Chas. Pfizer & Co. for a combination of tetracycline and oleandomycin is Signemycin.

We treated with tetracycline-oleandomycin 3 patients with chronic pneumonitis who were sent to us with a presumptive diagnosis of bronchogenic carcinoma, based on the presence of peripheral infiltrative lesions. *Staphylococcus aureus* and pneumococcus were isolated from the expectorate. The dosage used was 2 Gm. daily (500 mg. every six hours) for a week. After that period, we continued with 1 Gm. daily. Clinical improvement was rapid (one to two weeks), and it was accompanied by a marked reduction of the radiological image. Healing was obtained in one to two months, after the administration of 42, 38, and 53 Gm., respectively. We successfully treated pleural empyema after staphylococcal pneumonitis in a child aged 6 years; the dosage was 250 mg. every six hours for four days and then 250 mg. every eight hours for seven days. Three pleural punctures and aspiration of fluid were done. The patient received a total of 9 Gm. in 11 days.

We achieved the same effects, although not so dramatic, in the preoperative preparation of 2 patients with infected hydatid cysts of the lung, in whose expectorate was found Talamon Frenkel pneumococci. One Gm. of tetracycline-oleandomycin was administered daily. Operations were possible after two weeks. The

TABLE I  
Postoperative Use of Tetracycline-Oleandomycin\*

Pt.	Sex	Age, yr.	Diagnosis	Operation	Medication
1 N. D.	F	29	Pulmonary tuberculosis	7-27-57 thoracoplasty 5 ribs	1 Gm. daily, 7 days
2 G. R.	M	54	Bronchogenic carcinoma	10-2-57 left pneumonectomy	2 Gm. daily, 4 days
3 R. S.	M	50	Bronchogenic carcinoma	10-4-57 left upper lobectomy	1 Gm. daily, 8 days
4 E. B.	M	62	Bronchogenic carcinoma	10-26-57 left pneumonectomy	1 Gm. daily, 10 days
5 M. P.	M	29	Pulmonary tuberculosis	11-3-57 apicoposterior segmental resection	2 Gm. daily, 5 days
6 P. S.	F	41	Bronchiectasis after tuberculosis	11-10-57 left upper lobectomy	1 Gm. daily, 7 days
7 J. N.	M	14	Hydatid cyst	11-23-57 cystectomy	1 Gm. daily, 8 days
8 J. G.	F	27	Pleural empyema secondary to intrapleural rupture of hydatid cyst, pyogenic <i>Staph. albus</i>	11-21-57 decortication	2 Gm. daily, 14 days
9 C. B.	F	57	Neurogenic intrathoracic tumor	11-24-57 extirpation	1 Gm. daily, 6 days
10 B. S.	M	50	Bronchogenic carcinoma	12-26-57 right upper lobectomy	1 Gm. daily, 8 days
11 L. A.	M	62	Bronchogenic carcinoma	12-30-57 right pneumonectomy, mediastinal dissection	2 Gm. daily, 6 days
12 I. M.	F	27	Pulmonary tuberculosis	1-21-58 right upper lobectomy	1 Gm. daily, 8 days
13 A. O.	M	59	Bronchogenic carcinoma	2-7-58 left upper lobectomy	1 Gm. daily, 8 days
14 E. R.	F	30	Pleural empyema secondary to severe pneumonitis, pyogenic <i>Staph. albus</i>	2-13-58 decortication	1 Gm. daily, 10 days
15 A. M.	M	15	Hydatid cyst	2-15-58 cystectomy	1 Gm. daily, 7 days
16 R. A.	M	48	Bronchogenic carcinoma	3-3-58 right pneumonectomy, mediastinal dissection	2 Gm. daily, 5 days
17 R. G.	F	29	Pulmonary tuberculosis	4-17-58 left pneumonectomy	1 Gm. daily, 8 days
18 E. F.	F	46	Pulmonary tuberculosis	4-26-58 left pneumonectomy	2 Gm. daily, 4 days
19 H. V.	M	52	Bronchogenic carcinoma	5-19-58 left pneumonectomy	2 Gm. daily, 5 days
20 I. V.	F	26	Pulmonary tuberculosis	5-29-58 left pneumonectomy	1 Gm. daily, 8 days

\* Tolerance was excellent and results were good in every patient.

persistence of the original lesion delayed the total disappearance of the infectious phenomena. Less favorable results were obtained in the secondary infections of bronchogenic carcinoma. In the group of nonspecific pulmonary suppurations, we had favorable results in 2 cases of extended bilateral bronchiectasis, and a bout of severe infection was controlled in combination with postural drainage. The dosage was 1 Gm. daily for 10 days. In the expectorate of both patients was found *Staph. aureus*, nonhemolytic *Streptococcus*, and pneumococcus.

We wish to emphasize the result obtained with a very severe, fetid bronchopulmonary suppuration from which were isolated, by puncture of the abscess, pyogenic nonhemolytic *Streptococcus* and pneumococcus; both were sensitive to penicillin and streptomycin. Treatment based on 1,000,000 units and 1 Gm. daily (30 days) failed. A new study of expectorate revealed that the bacterial flora had changed and was now prevailing *Staphylococcus albus*, Talamon Frenkel pneumococcus, and coliform bacillus; all were sensitive to the tetracycline-oleandomycin combination and resistant to penicillin and streptomycin. We began treatment with 3 Gm. daily (500 mg. every four hours) for five days, and then 2 Gm. daily for 16 days. A great improvement was observed from the beginning. Expectoration diminished and lost its fetid character, hemoptysis and fever disappeared, appetite recovered, and general condition improved. After receiving 38 Gm., the patient had a slight glossitis, which subsided with local treatment and without withdrawal of the antibiotic. After a month, tetracycline-oleandomycin was interrupted because of the large amount administered (49 Gm.); after six days, expectoration increased, general condition worsened, and hemoptysis and fever reappeared. We resumed tetracycline-oleandomycin (250 mg. every four hours), restoring the patient to good surgical condition in two weeks. Right-side pneumonectomy was done. Tetracycline-oleandomycin was administered during the postoperative period, 1 Gm. intravenously for two days and 1.5 Gm. daily for 50 days. In spite of this, a month after operation, the patient had a serious wound sepsis and pleural empyema without a bronchopleural fistula (*Staph. aureus*). The late appearance of both complications permitted treatment under good general and local conditions, making possible pleural drainage, débridement of the wound, and finally thoracoplasty. The success of the oleandomycin-tetracycline combination, although limited to the preoperative period, was dramatic; in this case we had double proof, since the symptoms reappeared as soon as the antibiotic treatment was interrupted.

We have used tetracycline-oleandomycin in 5 patients with postoperative pleural empyemas, for which the antibiotic had not been used previously (table II). In 3 patients without bronchopleural fistula we obtained good results; in 1 of them, the oleandomycin-tetracycline had to be combined with intrapleural polymyxin B (pyocyanic and pyogenic *Staph. albus*). In the other 2 patients with bronchopleural fistula, the antibiotic was not effective. In 1, the infection remained until an intrathoracic foreign body (gauze) was removed. In the other, it was necessary to interrupt tetracycline-oleandomycin treatment because of intolerance reactions (glossitis and stomatitis) after administration of 21 Gm.; this also occurred with other antibiotics (penicillin, streptomycin, oxytetracycline). Healing was obtained with surgical drainage after a long course.

As an assay, we used tetracycline-oleandomycin as an adjuvant to sulfadiazine in a patient with severe pleuropulmonary suppuration due to *Actinomyces nocardia*; with this therapy and surgical drainage, we obtained a surprising immediate improvement. We administered tetracycline, 1 Gm. intravenously for eight days, with no side effects.

TABLE II  
Postoperative Empyema

Patient Sex Age, yr.	Diagnosis	Operation	Complication	Culture	Medication	Other therapeutic measures	Course	Results
1 B. P. M 22	Rib tumor (angioma)	10-8-57 pleuro- costal resection	Pleural empyema (no bronchopleural fistula)	Pyogenic <i>Staph.</i> <i>albus</i>	1 Gm. daily, 8 days; total 8 Gm.	Pleural drainage	Aporetic in 3 days	Good
2 J. V. M 22	Pulmonary tuberculosis	10-29-57 left pneumonectomy	Pleural empyema (no bronchopleural fistula), severe wound sepsis	Pyogenic <i>Staph.</i> <i>albus</i> , pyocyanic	2 Gm. daily, 7 days; 1 Gm. daily, 30 days; total 44 Gm.	Pleural puncture, polymyxin B	Aporetic in 12 days, pleural fluid sterile in 18 days	Good
3 M. E. F 21	Hypertensive pyopneumothor- acoplasty secondary to intrapleural rupture of hydatid cyst	12-18-57 decortication	Pleural empyema (with bronchopleural fistula)	<i>Staph.</i> <i>aureus</i>	1.5 Gm. daily, 10 days; 1 Gm. daily, 6 days; total 21 Gm.	Pleural drainage before antibiotic was used	No change	Poor, intolerance, glossitis
4 P. S. M 47	Bronchogenic carcinoma	4-10-58 right upper lobectomy	Pleural empyema (with bronchopleural fistula) intrapleural foreign body	<i>Staph.</i> <i>aureus</i>	1 Gm. daily, 9 days; total 9 Gm.	Pleural drainage, foreign body removed after antibiotic was used	No change	Poor
5 L. T. M 72	Bronchogenic carcinoma	4-7-58 left pneumonectomy	Pleural empyema (no bronchopleural fistula)	Pyogenic <i>Staph.</i> <i>albus</i>	1 Gm. daily, 20 days; total 20 Gm.	Pleural puncture	Aporetic in 3 days, pleural fluid sterile in 9 days	Good

Oral administration of tetracycline-oleandomycin is well tolerated, even when the drug is administered for a long time or in high dosages. Most of our patients received 10 to 15 Gm., 1 Gm. daily. In the severe case of pulmonary suppuration, the total dose was 140 Gm. during 3½ months of continuous administration; the patient had only a slight glossitis after receiving 38 Gm.; this disappeared without withdrawal of the antibiotic. Other patients have received large amounts of the antibiotic with good tolerance: 53 Gm. in 39 days, 44 Gm. in 36 days, 14 Gm. in 28 days.

In the only patient who received a dosage of 3 Gm. daily, for five days, the antibiotic was well tolerated; 2 Gm. daily has frequently been administered without harmful effects, even though the dose was continued for two weeks.

We have very little experience with this combination administered intravenously; only a few patients have received it, for 24 to 48 hours. In only 1 patient (with pleuropulmonary suppuration due to *A. nocardia*), we administered 1 Gm. daily for eight days (500 mg. dissolved in 500 ml. of 5 per cent dextrose solution every 12 hours). Tolerance was excellent.

We prepared our surgical patients with antibiotics. In the noninfected standard cases, lack of symptoms and data to determine bacterial flora, insufficiency of the method of study for determining the relationship between bacteria found in the expectorate and the pathological condition, the addition of other therapeutic agents that may modify the general and local conditions (rest, transfusion, postural drainage) make it difficult to evaluate efficiency of the drug.

We consider that tetracycline-oleandomycin was of value in this group, as demonstrated by the decrease or disappearance of the tracheobronchial secretions. In 2 patients with low fever, temperature became normal after 48 and 72 hours of treatment, respectively.

Antibiotics are always indicated during the first days after a thoracic surgical procedure. Their use is justified because of the wide incisions, the large number of hemostatics and sutures required, which may favor bacterial growth, and the handling of infected tissues. It is difficult to establish the degree of protection provided by a particular antibiotic. Even so, we believe that tetracycline-oleandomycin was effective in our series of 20 patients, in which no complications were recorded. This is confirmed by decorticated pleural empyemas, in which it was impossible to avoid contamination of the wound during surgery and is even more evident, since both patients were operated on at a time when our operating suite was being repaired, during which period we noticed an increase of wound sepsis.

From the therapeutic aspect, tetracycline-oleandomycin was valuable in the treatment of chronic pneumonitis, pleural empyema without bronchial fistula, and chronic bronchopulmonary suppuration and in stopping infectious bouts of extended bronchiectasis. It was effective, although less so, in the preoperative preparation of patients with infected hydatid cysts and infectious phenomena that accompany bronchogenic carcinoma. It was useful, also, as an adjuvant to sulfadiazine in 1 case of pleuropulmonary suppuration due to *A. nocardia*.

We observed here, as in other surgical media, a recrudescence of the severity and number of postoperative infections. Contamination occurs during surgery; in our series the bacterial flora of wound infections was, in general, different from bronchial and esophageal flora.

*Staphylococcus* was the most common agent, being present in 100 per cent of

the cases we studied. It is frequently resistant to penicillin, still the most used antibiotic. This is the reason for our interest in the broad-spectrum antibiotics and in combinations such as tetracycline-oleandomycin. We emphasize the fact that we have not found resistant strains to this antibiotic. We do not know what will happen when its use is generalized, but the limited number of strains resistant to tetracycline led us to believe that its combination with oleandomycin would be even more effective.

#### CONCLUSIONS

1. Tetracycline-oleandomycin is an effective antibiotic combination when used as a prophylactic medium, in patients who are to have pleuropulmonary operations, and in the postoperative period. It is also active in the treatment of severe infectious bronchopulmonary lesions and its severe postoperative complications, when infection is an important factor.
2. The drug is well tolerated even when high dosages are administered for a long period.
3. In the group of bacteria sensitive to this antibiotic, we have not found resistant strains.

# Actual Therapeutic Approach to the Treatment of Osteomyelitis

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The frequent occurrence of pyogenic infections in osteomyelitis has instigated intensive investigation of the problem. The bone, a hard, resistant, unmalleable material having a scanty blood supply, acts as a fortress of few defenses when confronted with an infectious process in its interior. It reacts with limited measures, and permits the pathogen not only to destroy the osseous element, but also to invade surrounding tissue, carrying the infection to softer parts and producing fistulae that manifest an indolent pathogenicity, thus giving osteomyelitic infections a definite tendency to chronicity and refractoriness to many types of treatment. In the course of our experience with 1568 cases from 1940 to 1958, we have utilized various suggested approaches, proving, in some cases with hope and in others with despair, the efficacy of the therapy in question.

Two basic elements specifically condition such cases: the pyogenic organism and the biologic defenses of the patient. Since the pyogenic organism is the root cause of osteomyelitis, we direct our major therapeutic effort against it in order to destroy it. Sensitivity tests showed that the organism usually found in the cases that we treated was a coagulatory pathogenic *Staphylococcus*, sensitive to an oleandomycin-tetracycline combination.\* Twenty-five cases of various types of osteomyelitis were treated over a period of one and a half years; brief histories of the principal cases follow.

A. L. Osteomyelitis of the lower third of the left femur of three years' duration was present. Radiography showed sequestra. For a period of three years prior to operation on February, 1957, there had been profuse purulent discharge and elimination of sequestra. Therapy, consisting of oleandomycin-tetracycline and blood transfusions, was initiated in April, 1957; supuration continued until a total of 14 Gm. of the antibiotic combination had been administered orally. The process ceased and the patient was then treated as an ambulatory case. Antibiotic therapy was discontinued at this stage; the patient was given only isoniazid and blood transfusions. The new antibiotic therapy (oleandomycin-tetracycline) improved the clinical picture markedly, halted suppuration. The case progressed favorably from the radiographic point of view, but the suppurative process was renewed when the drug was withdrawn.

L. T., aged 12, had osteomyelitis of the first metatarsal bone and proximal phalanx of the left foot. The symptoms began in January, 1957, and the patient was admitted to the hospital in March with the process advanced to the point at which there was total destruction of the first metatarsal bone, left foot, and the proximal phalanx of the same foot, with profuse purulent discharge. We intervened surgically, filling the infected cavity with fresh bone, removed the day before from another patient. Treatment with oleandomycin-tetracycline, at the rate of 750 mg. daily, in conjunction with blood transfusions was initiated. The case responded admirably to the treatment of choice, that is, filling the infected cavity, broad-spectrum antibiotics, blood transfusions, and supportive medication. The total of antibiotics administered was 12 Gm. The lesion has healed completely.

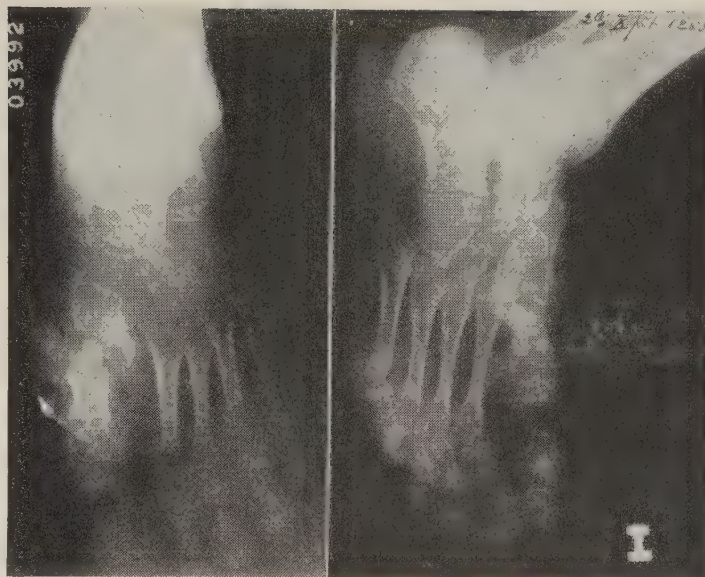
A. B., aged 12, was admitted to the hospital in March, 1957, with osteomyelitis of the left tibial diaphysis and the upper third of the right humerus. Infection was two years' duration. Surgical measures were taken only on the tibia, but both foci progressed simultaneously toward

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\* The trade name of Chas. Pfizer & Co. for the oleandomycin-tetracycline combination is Signemycin.

FIG. 1. (Left) Osteomyelitis in the first metatarsal and first cuneiform bone is shown before treatment with tetracycline-oleandomycin combination.

FIG. 2. (Right) This is the same foot after treatment with tetracycline-oleandomycin combination.



sclerosis with improvement in the over-all status. Oleandomycin-tetracycline was administered, 12.5 Gm., with a favorable response. Patient was discharged from the hospital with lesions healed (figs. 1 and 2).

E. Q., aged 12, had osteomyelitis of the right tibia. Admitted to the hospital for the first time in June, 1955, presenting sequestra, and he was operated upon. Osseous repair by graft of the tibia and graft-filling with stored bone. Patient had had treatment with various antibiotics with frequent relapses until the administration of oleandomycin-tetracycline. After administration of 10 Gm. of this combination there was ostensible clinical improvement, verified by radiography. At present no fistula is suppurating and deformation of the foot is markedly improved (figs. 3, 4).

S. P., aged 12, presented with osteomyelitis of the right tibia. The process was of long duration with almost the entire right tibia sequestered, with osteomyelitis of the sternum and right clavicle also. For a period of more than a year the patient had received treatment with

FIG. 3. (Left) Shown is osteomyelitis in the right tibia before treatment.

FIG. 4. (Right) This is the right tibia after treatment with tetracycline-oleandomycin combination.



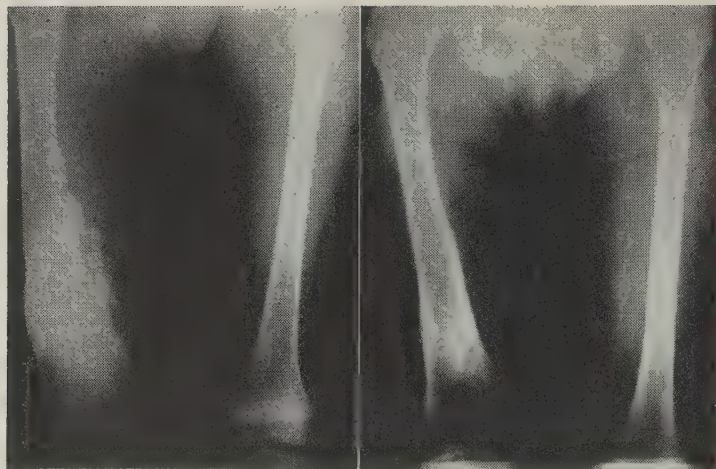


FIG. 5. (Left) Illustrated is case of osteomyelitis in the right femur prior to tetracycline-oleandomycin treatment.

FIG. 6. (Right) This roentgenogram shows the right femur after treatment with tetracycline-oleandomycin combination.

the usual antibiotics without visible improvement in the infectious process. In June, 1957, 10 Gm. of oleandomycin-tetracycline were administered. The patient improved markedly; the patient considered himself cured, abandoned the hospital, and discontinued treatment. Therapy had to be reinstituted and at present, the case is much improved, the sequestrum having been eliminated almost spontaneously. The lesion has closed and treatment with the antibiotic combination, transfusions, and antipyogenic vaccine is continuing.

J. C., aged 12. After the operation, for osteomyelitis of the right femur, performed July 15, 1957, in which the sequestra were removed, two active fistulae, with copious suppuration, appeared, one in lateral-internal portion of the right muscle, the other in the lower posterior. These were associated with considerable deformity. On July 22, 1957, four days after the initiation of oleandomycin-tetracycline therapy, the suppuration had diminished by 70 per cent, with only 2 Gm. of the antibiotic combination administered. Blood transfusions begun. On August 13, 1957, after a total of 6 Gm. of the antibiotic combination had been administered, one of the fistulae was cured. On December 11, 1957, both fistulae closed and radiographic examination showed marked osseous regeneration. The patient's present condition is excellent and the most recent roentgenograms showed notable restoration. The total of oleandomycin-tetracycline administered was 20 Gm. This patient, prior to present treatment, had received sulfonamides, penicillin, streptomycin, and chloramphenicol in elevated dosages (figs. 5 and 6).

## RESULTS

In the 25 cases treated with the oleandomycin-tetracycline combination it was observed that patients generally showed a decrease in suppuration within a few days after commencement of treatment, with cessation of suppuration at a later date, and that the fistulae assume a healthier condition before being completely cured.

The causal bacterial strains in these types of cases, according to sensitivity tests, are generally staphylococci, being mainly the highly pathogenic *Micrococcus pyogenes* var. *aureus*. Post-therapeutic radiographic examination in most cases showed frank osseous regeneration.

It was observed in some cases that when the administration of oleandomycin-tetracycline was discontinued, the suppurative process was renewed. Upon reinstitution of the antibiotic combination, frank improvement was noted.

The methods of administration were oral and intravenous. Tolerance was good. In our therapy we employed a combination of antibiotic treatment, blood transfusions, vaccines, sequestrectomy, and filling of cavities with homologous bone.

Even though chronic osteomyelitis occurs frequently, patients in the acute stage

of the infection applying for hospital admission are not common; our experience in this respect leads us to believe that the broad spectrum antibiotics do not halt or eradicate the infection, but permit it to progress to chronicity, with the result that osseous lesions are much more reduced and localized, thus permitting more rapid and efficacious cure with the above mentioned therapy.

#### SUMMARY AND CONCLUSIONS

Six, out of a total of 25 clinical cases, which have been treated according to the therapy set forth herein, together with radiographic data, are presented.

The response noted within a few days after initiation of therapy shows diminution of suppuration and improvement of fistulae, with clinically favorable responses being obtained in a relatively short time. Especially noteworthy is the good toleration of the oleandomycin-tetracycline combination. It is notable that in nearly all cases treated, other antibiotics had previously been administered without satisfactory results.

Bacteriological studies demonstrate the efficacy of the antibiotic combination against pathogens that have become resistant to other preparations.

# Genitourinary Infections Treated with the Antibiotic Combination Tetracycline-Oleandomycin

HECTOR SCHENONE

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The difficulty of clinical treatment of urogenital infections led us to try the antibiotic combination tetracycline-oleandomycin\* for these cases and especially in those patients with microorganisms resistant to other antibiotics or who had already been treated without any result.

## METHOD AND PROCEDURE

A series of 30 patients with urinary infections have been treated with the tetracycline-oleandomycin combination. Generally we were dealing with patients with pyuria and in whom bacteriological identification showed the presence of *Micrococcus pyogenes* var. *aureus*, *Aerobacter aerogenes*, *Escherichia coli*, enterococcus, *Proteus* species, and *Pseudomonas aeruginosa*.

The treatment given consisted of 1 Gm./day, divided into four capsules of 250 mg. each. Only on rare occasions was it necessary to begin treatment with 1.5 Gm. four times a day. Sensitivity tests were made using the disc method, as it was the easiest and most convenient one.

## RESULTS

In table I are shown the clinical results obtained with this treatment, diagnosis, causative agent, as well as other information of interest.

The therapeutic response in these 30 patients was rated as follows: excellent in 13 cases, satisfactory in 13, fair in 2, and no response in 2. In the 2 patients failing to respond, the offending organism was resistant *Ps. aeruginosa*. Those cases in which the results were only fair showed reduction in pyuria despite failure to sterilize the urine completely. Generally, these results were obtained on the fifth or sixth day of treatment, only rare cases necessitating longer treatment.

## TOLERANCE

Tolerance of the antibiotic combination tetracycline-oleandomycin was generally excellent. Only 3 patients displayed minor inconveniences (glossitis, mild diarrhea, anal pruritus), but these did not give rise to any sequelae and it was not necessary to suspend treatment.

## DISCUSSION AND CONCLUSIONS

The most difficult cases of infection in urological practice were treated with the antibiotic combination tetracycline-oleandomycin; the drug was effective in the majority of cases. A great many of our patients had already been treated previously with other antibiotics without any result.

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\* The trade name of Chas. Pfizer & Co. for the combination oleandomycin-tetracycline is Signemycin.

TABLE I

*Genitourinary Infections Treated with the Antibiotic Combination Tetracycline-Oleandomycin:  
Clinical and Bacterial Effectiveness*

Case	Pt.	Diagnosis	Organisms	Response*	Previous treatment	Comments
1	P. N.	Subacute urethritis	<i>Staphylococcus</i> , nonpathogenic, <i>Corynebacterium</i>	Satisfactory	None	Persistence of urine filaments
2	A. H.	Subacute urethroph prostatitis	<i>Staphylococcus</i> , nonpathogenic	Satisfactory	Penicillin, strep- tomycin, tetracycline	—
3	J. C.	Urethroph prostatitis	<i>E. anaerogenes</i> , <i>Staphylococcus</i> , nonpathogenic	Satisfactory	Sulfisoxazole	—
4	O. M.	Subacute urethritis	<i>Staphylococcus</i> , nonpathogenic	Fair	Penicillin, streptomycin	—
5	L. U.	Subacute urethritis	<i>E. coli</i>	Satisfactory	Chlortetracycline, novobiocin	—
6	T. J.	Subacute urethritis	<i>Staphylococcus</i> , nonpathogenic	Excellent	Penicillin, streptomycin	—
7	J. F.	Subacute urethritis	<i>Staphylococcus</i> <i>albus</i> , nonpathogenic	Excellent	Penicillin, sulfisoxazole	—
8	J. P.	Pyelocystitis	<i>Proteus vulgaris</i>	Excellent	None	Surprising because of the type of mi- crobe; cure was kept under control
9	S. P.	Subacute urethritis	<i>M. pyogenes</i> var. <i>aureus</i>	Excellent	Penicillin, streptomycin, sulfisoxazole	—
10	J. R.	Chronic urethroph prostatitis	<i>M. pyogenes</i> var. <i>albus</i>	Satisfactory	Sulfisoxazole	Persistence of small urethral discharge
11	M. Z.	Pyelocystitis	<i>E. intermedium</i>	Excellent	None	—
12	J. A.	Subacute urethritis	<i>M. pyogenes</i> var. <i>albus</i>	Satisfactory	None	—
13	J. S.	Acute urethritis	<i>M. pyogenes</i> var. <i>albus</i>	Satisfactory	Penicillin, sulfisoxazole	—
14	H. I.	Pyelocystitis	—	Excellent	None	—
15	M. F.	Acute cystitis	<i>E. coli</i>	Excellent	Sulfisoxazole	—
16	A. C.	Urethroph prostatitis	<i>M. pyogenes</i> var. <i>albus</i>	Satisfactory	Sulfisoxazole	Mild glossitis
17	L. P.	Subacute urethritis	<i>M. pyogenes</i> var. <i>albus</i>	Fair	None	Persistence of morn- ing urethral dis- charge
18	L. C.	Acute cystitis	<i>E. coli</i>	Excellent	None	—
19	C. K.	Cystitis	<i>Staphylococcus</i> , nonpathogenic	Excellent	None	—
20	L. A.	Cystitis, vesical lithiasis	<i>E. anaerogenous</i>	Satisfactory	Sulfisoxazole,	Remarkable im- provement of symp- toms
21	A. N.	Acute prostatitis, epididymitis	<i>E. coli</i>	Excellent	None	Mild diarrhea
22	A. F.	Lithiasic pyelonephritis	Enterococcus	Satisfactory	None	Remarkable im- provement of symp- toms
23	A. C.	Renal lithiasis, pyuria	<i>E. coli</i>	Satisfactory	None	—
24	M. L.	Pyuria after adenectomy	<i>Ps. aeruginosa</i>	Failed	Penicillin, streptomycin	—
25	V. P.	Prostatitis after catheterism	<i>Staphylococcus</i> , nonpathogenic	Satisfactory	None	Temperature normal after 48 hr. of treat- ment
26	G. F.	Pyuria after prostatectomy	<i>E. coli</i> , <i>Staphylococ-</i> <i>cus</i> , nonpathogenic	Satisfactory	Penicillin, streptomycin, tetracycline	Anal pruritus

*Table I Continued on Page 318*

TABLE I (Continued)

*Genitourinary Infections Treated with the Antibiotic Combination Tetracycline-Oleandomycin:  
Clinical and Bacterial Effectiveness*

Case	Pt.	Diagnosis	Organisms	Response*	Previous treatment	Comments
27	P. M.	Cystitis	<i>E. coli</i> , <i>Staphylococcus</i> , nonpathogenic	Excellent	Sulfisoxazole, sulfonamides, & penicillin plus streptomycin	—
28	E. V.	Pyuria after prostatectomy	<i>Ps. aeruginosa</i>	Failed	Nitrofurantoin, tetracycline, penicillin, oxytetracycline, streptomycin	—
29	R. C.	Pyelocystitis	<i>A. aerogenes</i> , <i>Staphylococcus</i> , nonpathogenic	Excellent	—	—
30	J. E.	Acute epididymitis, prostatitis	—	Excellent	—	—

\* Excellent: When the patient was cured and follow-up did not show any relapse; satisfactory: when results permitted the patient to return to his daily activities, even though a slight discomfort still remained, which took some time before disappearing completely; fair: when there was a noticeable decrease of the symptoms, especially with respect to pyuria, in spite of the failure to sterilize the urine completely.

In conclusion, this antibiotic combination was clinically effective in urinary tract infections, and its tolerance was excellent with no side effects at all with the dosage employed.

## BIBLIOGRAPHY

1. GROVE, D. C., AND RANDALL, W. A.: *Assay Methods of Antibiotics: A Laboratory Manual*, New York, Medical Encyclopedia, Inc., 1955.
2. LOUGHLIN, E. H., AND MULLIN, W. G.: Combined antibiotic therapy of tropical infections with oxytetracycline-oleandomycin and tetracycline-oleandomycin. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 698.
3. FIORA, F.: Sigmamycin in acute blennorrhagia, *Dia méd.* 30:570, April 3, 1958.
4. CHIARENZA, A.: Observations on the effects of Sigmamycin in non-gonococcal urethritis, *Minerva med.* 48:2692, 1957.
5. DATES, J. K.: Trial of Sigmamycin in non-specific urethritis, *Brit. J. Ven. Dis.* 34:38, 1958.
6. WILLCOX, R. R.: Tetracycline and oleandomycin in combination in nongonococcal urethritis. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 672.
7. REVELLI, E., AND DURANDO, C.: Treatment of the specific inflammatory component of tuberculosis of the female genital organs, *Minerva med.* 48:2658, 1957.
8. ARRIGONI, G.; GRIGNANI, G. C., AND VARESI, M.: A new antibiotic association in the treatment of urologic infections, *Minerva med.* 48:2701, 1957.

# Triacetyloleandomycin in the Therapy of Pediatric Infections

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During the past year, a revival of interest in oleandomycin has occurred, due to the development of a new salt, triacetyloleandomycin. This preparation has been demonstrated to give blood concentrations superior to the oleandomycin phosphate originally available.<sup>3</sup> Because of the superior absorption of triacetyloleandomycin, it was felt that a clinical trial was indicated to determine its effectiveness in the management of pediatric infections; in addition, a study to confirm the pharmacological differences of the oleandomycins was also indicated. Additional interest for this study was found in the increasing resistance to erythromycin of isolated strains of staphylococci from infections treated in a large urban hospital.

## METHODS

In the clinical trial, 40 children, considered in need of antibacterial therapy by the house staff, were given an oral suspension of triacetyloleandomycin after appropriate diagnostic studies at the time of admission; the suspension, containing 125 mg./5 ml., was administered in doses of approximately 10 mg./lb. The dose was repeated every six hours until the disease responded or therapy was changed, because of apparent therapeutic failure. The only criterion for eliminating patients from this study during the trial period was the existence of an infection that was deemed life-threatening to the child should effective therapy be delayed.

In many cases, serum was obtained from these patients for the bioassay of oleandomycin content, in addition to routine laboratory procedures.

Occasionally, children were continued on the therapy in effect prior to admission, in addition to the drug under study.

The laboratory study included a pharmacological study of absorption of triacetyloleandomycin in healthy volunteer adults as well as serum concentrations in many of the children treated in the clinical studies. Sensitivity studies were done on selected staphylococcal isolates to confirm the effectiveness of this agent in some of the infections caused by staphylococci.

A triple crossover study was carried out in 30 healthy adult subjects; each subject received one of three different preparations on each of three test days. Oleandomycin phosphate in capsules, triacetyloleandomycin in capsules, and erythromycin, in tablet form as the stearate or as crystalline material, were administered in single 250 mg. doses to the subjects in the fasting state; blood samples were collected by venipuncture prior to administration of the preparations and at one, two, three, and six hours afterward. Total urinary collections were also made for 24 hours. Assay of both serum and urine samples for content of the proper antimicrobial agent were carried out by a cup plate bioassay method employing *Sarcina lutea* as the test organism.

One or more blood samples were collected from children on treatment schedules of triacetyloleandomycin, and the sera from these bloods were similarly tested for antimicrobial activity. Samples were collected at two, four, or six hours following doses, so as to gain information on clinical blood levels.

TABLE I

Clinical Results with Triacetyloleandomycin

Clinical diagnosis	No. pt.	Age, yr.		Triacetyloleandomycin, mg./dose		Predominating bacteria	Patient responses, %			
		Range	Mean	Range	Mean		Drug alone	Drug alone after failure of other chemotherapy	Drug combined with other chemotherapy	Total response
Osteomyelitis	1	7		10		Hemolytic <i>Staph. aureus</i>		1/1 (100)	1/1 (100)	1/1 (100)
Tonsillitis, pharyngitis	9	1 1/2-7	3.7	2.5-10	8.3	Hemolytic <i>Staph. aureus</i> , $\alpha$ -hemolytic <i>Streptococcus</i> , <i>Neisseria</i>	4/5 (80)	1/1 (100)	2/3 (66.7)	7/9 (77.8)
Pneumonia	11	1/2-7	2.3	2.7-11	9.1	Hemolytic <i>Staph. aureus</i> , $\alpha$ -hemolytic <i>Streptococcus</i> , <i>H. influenzae</i> , <i>Pseudomonas</i>	4/5 (80)	3/4 (75)	2/2 (100)	9/11 (81.7)
Pyelonephritis	2	2/12-4 1/2	2.3	9-10	9.5	<i>Aerobacter</i> , <i>Pseudomonas</i>		0/2 (0)		0/2 (0)
Enteritis	1	4/12		10			1/1 (100)			1/1 (100)
Abscess (subcutaneous)	2	3/12-9	4.6	4.2-11.0	7.6	Hemolytic <i>Staph. aureus</i>	1/1 (100)		1/1 (100)	2/2 (100)
Cervical adenitis	1	2/12		9.3			1/1 (100)			1/1 (100)
Otitis media	3	1 4/12-3	2.4	9.4-10	9.8	Pneumococci	2/2 (100)		1/1 (100)	3/3 (100)
Bronchiolitis	7	1/12-1 4/12	6/12	9.4-12.0	10.4	Hemolytic <i>Staph. aureus</i> , <i>E. coli</i>	2/2 (100)	1/1 (100)	4/4 (100)	7/7 (100)
Pyoderma	3	6/12-7	3.0	3-10	7.7	Hemolytic <i>Staph. aureus</i> , $\beta$ -hemolytic <i>Streptococcus</i>	3/3 (100)			3/3 (100)
Total % response							18/20 (90)	5/8 (62.5)	11/12 (91.5)	34/40 (85)

TABLE II  
*Bacteriology of Infections Treated*

Infection	No. of effective cases	No. of failure cases
<i>Micrococcus pyogenes</i> var. <i>aureus</i>	12	3
Streptococci	5	0
Pneumococci	4	0
<i>Aerobacter aerogenes</i>	0	2
<i>Pseudomonas</i>	(1)	3
<i>Hemophilus influenzae</i>	0	1

Fifty selected staphylococci isolated from clinical infections were also studied by a twofold tube dilution technique for the minimal inhibiting concentration of oleandomycin and erythromycin; tests for residual viable organisms were made by plating the broths of all tubes showing inhibition of the test organism.

### RESULTS

The children treated spanned the general pediatric population, with ages ranging from 1 month to 7 years old. The dose of the drug, based on body weight, was usually 10 mg./lb. The results of the clinical trials are summarized in table I.

Reaction to therapy was considered favorable if fever abated permanently within 48 hours of the start of the antibiotic, in association with general clinical improvement. If a patient was subsequently started on another chemotherapeutic agent for the infection under study, regardless of the temperature response, this represented a treatment failure. The over-all therapeutic effectiveness was 85 per cent, as 34 of the 40 infections remitted permanently, in accordance with the stipulated criteria of favorable drug reaction.

The response pattern was further analyzed in terms of associated chemotherapy into three groups: (1) those previously untreated infections for which triacetyloleandomycin was the only administered agent; (2) therapeutic failures with other established antimicrobials, which were subsequently treated with triacetyloleandomycin alone; and (3) a combined therapy group, in which either the chemotherapeutic agents used before were inadequately tested and cannot be considered treatment failures or the therapeutic regimen was that of triacetyloleandomycin combined with other drugs. The per cent responses of the three groups are 90, 62.5, and 91.5 respectively, with the poorest response noted in the group of treatment failures with other agents given before. In several of this group of failures, there was clinical evidence that an underlying nonbacterial infection was operative.

Table II lists those infections from which a predominating bacterial agent could be identified. Striking responses were noted in infections associated with staphylococci, pneumococci, and streptococci. Eight of the 12 patients with staphylococcal infections responding to therapy received triacetyloleandomycin alone, 2 received the drug combined with chloramphenicol, and 1 each received penicillin and a sulfonamide as adjunctive treatment.

Bacteriological follow-up was not obtained in all cases. The same organisms persisted in the urine of 2 cases of *Aerobacter* and *Pseudomonas* infection; in 1 case of pneumonia, *Hemophilus influenzae* predominated in the nasopharyngeal flora both before and after seven days of therapy.

TABLE III  
*Serum Concentrations and Urinary Recoveries*  
*Following 250 mg. Triacetyloleandomycin, Oleandomycin Phosphate, and Erythromycin*  
*Given Orally in the Fasting State*

Subject	Date	Serum concentrations, $\mu\text{g.}/\text{ml.}$ Hours after administration					Urinary recovery			
		Control	1	2	3	6	Urine vol., ml.	Urine conc., $\mu\text{g.}/\text{ml.}$	Total, $\mu\text{g.}/\text{ml.}$	Per cent recovery
Triacetyloleandomycin										
Bl	12/20	0	.53	.72	.36	<.1	2960	11.5	34040	13.6
Br	12/17	0	.26	.24	.15	<.1	1370	16.5	22605	9.0
Co	12/17	0	<.1	1.23	.92	.27	1195	54.0	64530	25.8
Du	12/16	0	.45	.32	.21	.11	1650	22.0	36300	14.5
Fe	12/17	0	<.1	<.1	<.1	<.1	690	3.4	2346	.9
Fra	12/17	0	<.1	<.1	<.1	<.1	870	9.8	8526	3.4
Frd	12/16	0	.27	.32	.23	.11	780	31.0	24180	9.7
Fre	12/17	0	.68	.69	.54	.12	915	36.0	32940	13.2
Ge	12/16	0	.10	.42	.30	.15	900	22.0	19800	7.9
Gi	12/12	0	.78	.62	.40	.12	2040	15.5	31620	12.6
Gra	12/12	0	.42	1.12	1.74	.42	2000	33.5	67000	26.8
Gre	12/13	0	1.80	1.32	.90	.44	450	115.0	51750	20.7
Grn	12/12	0	.81	1.05	1.10	.31	650	93.0	60450	24.2
Ha	12/12	0	.66	.64	.45	.21	1105	41.0	45305	18.1
Ham	12/13	0	<.1	1.04	.90	.34	1135	47.0	53345	21.3
He	12/17	0	<.1	.79	.60	.17	1255	26.0	32630	13.1
Ko	12/12	0	.61	1.14	.98	.37	955	29.5	28172	11.3
Li	12/20	0	.72	.48	.40	.13	1115	27.0	30105	12.0
Lo	12/13	0	.44	1.44	1.06	.32	1415	43.0	60845	24.3
Lor	12/20	0	.54	.67	.63	.36	1310	42.0	55020	22.0
Ma	12/13	0	.43	.90	1.04	.44	810	62.0	50220	20.1
Mi	12/13	0	1.59	1.12	.93	.32	1795	31.0	55645	22.3
Ra	12/19	0	.57	.92	.67	.18	970	56.0	54320	21.7
Ras	1/7/58	0	1.60	1.13	.57	.34	825	76.0	62700	25.1
Ro	12/19	0	.48	1.15	.78	.34	850	56.0	47600	19.0
Rod	12/20	0	.32	1.14	.75	.22	1070	48.0	51360	20.5
Ru	12/20	0	.17	1.10	.78	.36	2195	28.0	61460	24.6
Sa	12/19	0	.40	.41	.59	.15	1530	22.0	33660	13.5
St	12/20	0	<.1	.11	.18	.34	1210	42.0	50820	20.3
Wa	12/20	0	.38	.78	.52	.22	880	36.0	31680	12.7
Av.			.50	.77	.62	.23				17.0
Oleandomycin phosphate										
Bl	12/13	0	<.1	.72	.31	.13	1900	8.0	15200	6.1
Br	12/14	0	.17	.43	.24	<.1	1045	16.0	16720	6.7
Co	12/14	0	<.1	.40	.41	.21	960	18.5	17760	7.1
Du	12/12	0	.27	.26	.59	.16	1335	16.0	21360	8.5
Fe	12/14	0	.18	.19	.81	.25	780	20.0	15600	6.2
Fra	12/12	0	.19	1.20	.75	.17	1140	21.5	24510	9.8
Frd	12/12	0	.14	.90	.51	.21	965	27.0	26055	10.4
Fre	12/14	0	.16	.27	.35	.17	1035	12.0	12420	5.0
Ge	12/12	0	.14	.91	.50	.23	595	5.1	3034	1.2
Gi	12/19	0	.19	.57	.43	.20	1390	12.0	16680	6.7
Gra	12/19	0	.14	.57	.34	.18	1085	21.5	23328	9.3
Gre	12/20	0	<.1	.34	.44	.42	1310	18.5	24235	9.7
Grn	12/19	0	.19	.12	1.08	.26	925	16.5	15262	6.1
Ha	12/19	0	<.1	.16	.33	.22	1250	33.0	41250	16.5
Ham	12/20	0	.11	<.1	.63	.19	865	24.5	21192	8.5
He	12/13	0	.28	.80	.56	.13	1195	35.0	41825	16.7
Ko	12/19	0	.51	1.20	.66	.15	685	34.0	23290	9.3
Li	12/17	0	.20	.14	.17	.15	1260	17.5	22050	8.8
Lo	12/20	0	<.1	.78	.48	.19	1185	6.4	7584	3.0
Lor	12/17	0	.37	.30	.60	.24	1130	20.5	23165	9.3
Ma	12/20	0	<.1	<.1	.44	.40	1175	23.0	27025	10.8
Mi	12/20	0	<.1	.12	.13	<.1	795	4.6	3657	1.5
Ra	12/16	0	.27	.21	.34	.30	915	20.0	18300	7.3

*Table III Continued on Page 323*

TABLE III (Continued)  
*Serum Concentrations and Urinary Recoveries*  
*Following 250 mg. Triacetyloleandomycin, Oleandomycin Phosphate, and Erythromycin*  
*Given Orally in the Fasting State*

Subject	Date	Serum concentrations, $\mu\text{g.}/\text{ml.}$ Hours after administration					Urinary recovery			
		Control	1	2	3	6	Urine vol., ml.	Urine conc., $\mu\text{g.}/\text{ml.}$	Total, $\mu\text{g.}/\text{ml.}$	Per cent recovery
Ras	12/20	0	.27	.30	.81	.38	1280	14.0	17920	7.2
Ro	12/16	0	.22	1.09	.71	.38	640	40.0	25600	10.2
Rod	12/17	0	<.1	.11	.11	.14	975	12.5	12188	4.9
Ru	12/17	0	<.1	.93	.57	.16	750	32.0	24000	9.6
Sa	12/16	0	<.1	.14	.44	.11	1010	23.0	23230	9.3
St	12/17	0	.47	.69	.37	.16	860	21.0	18060	7.2
Wa	12/17	0	.17	.37	.89	.47	780	24.0	18720	7.5
		Av.	.15	.48	.50	.21				8.2
Erythromycin stearate										
Bl	1/7/58	0	.22	.29	.16	<.05	1225	1.25	15313	.61
Br	12/20	0	<.05	1.30	.42	.14	975	2.90	28275	1.10
Co	12/20	0	<.05	.73	.43	.20	695	5.63	39094	1.60
Fe	12/20	0	.31	.22	.62	.19	1010	5.80	58580	2.30
Fre	12/20	0	.18	.70	.29	.06	1340	3.40	45560	1.80
Gi	12/15	0	.13	.48	.32	.12	1340	3.65	48910	1.95
Gra	12/16	0	<.05	.08	.08	<.05	1575	.16	2520	.10
Gre	12/16	0	<.05	.11	.27	<.05	1070	1.04	11128	.45
Ha	12/16	0	<.05	.07	.34	.10	1555	1.25	19438	.78
Ko	12/16	0	.11	.83	.49	.14	1020	5.38	54825	2.20
Li	12/12	0	<.05	<.05	.74	.26	1140	11.25	12825	5.10
Lor	12/12	0	.21	1.30	.61	.16	1105	6.63	73206	2.90
Ra	12/12	0	.19	.85	.81	.09	835	7.13	59494	2.40
Ro	12/12	0	.13	.43	.43	.15	580	.92	53360	.21
Sa	12/12	0	.11	.27	.20	.08	1150	1.88	21620	.86
		Av.	.11	.51	.41	.12				1.67
Erythromycin capsules										
Du	12/19	0	<.05	1.10	.95	.26	1425	7.50	10688	4.30
Fra	12/20	0	<.05	<.05	.37	.25	960	6.63	63600	2.50
Frd	12/19	0	<.05	.12	.35	.14	795	5.13	40754	1.60
Ge	12/19	0	<.05	3.35	1.52	.26	685	11.25	77063	3.10
Ham	12/17	0	<.05	.27	1.52	.19	910	8.38	76213	3.00
He	12/20	0	<.05	.62	.37	.11	1065	4.50	47925	1.90
Lo	12/17	0	.63	.77	.54	.16	1250	9.00	11250	4.50
Ma	12/17	0	<.05	<.05	<.05	.53	1090	3.20	34880	1.40
Mi	12/17	0	.12	.73	.53	.18	1265	2.80	35420	1.40
Ras	12/14	0	<.05	2.50	1.75	.24	1310	6.75	88425	3.50
Rod	12/14	0	<.05	<.05	.11	.17	1250	1.90	23750	.95
Ru	12/14	0	<.05	.37	.47	.19	1735	2.35	40773	1.60
St	12/14	0	.08	.41	.35	.21	1135	8.60	97610	3.90
Wa	12/14	0	<.05	<.05	.46	<.05	1085	9.13	99006	3.96
		Av.	.06	.69	.65	.20				2.60

The average duration of therapy was 7.4 days, with a range of 3 to 31 days. No adverse hematological reactions were noted during therapy. The drug was discontinued for reasons of possible toxicity in three instances (7.5 per cent). In 1 patient, a macular rash developed after 31 days of combined chloramphenicol-triacetyloleandomycin therapy, which subsided within 48 hours of stopping the two drugs. In the other 2 children, vomiting was the symptom responsible for cancelling further medication; however, in 1 of the 2, the drug was reinstituted after 48 hours with no recurrence of the apparent gastric intolerance. There was no sign of bowel intolerance in terms of diarrhea secondary to this medication.

During the treatment of 21 patients, blood samples were collected at various times in relation to the dosage schedule. Serum concentrations of oleandomycin

TABLE IV

*Comparative Study of Oleandomycin, Triacetyloleandomycin, and Erythromycin in 30 Men—  
Average Serum Concentrations,  $\mu\text{g./ml.}$ , Following 250 mg. Oral Dose*

	Hours after administration				Total 24 hour urinary excretion, per cent
	1	2	3	6	
Oleandomycin	.15	.48	.50	.21	8.2
Triacetyloleandomycin	.50	.77	.62	.23	17.4
Erythromycin stearate	.11	.51	.41	.12	1.7*
Erythromycin capsules	.06	.69	.65	.20	2.6†

\* Average of 14 men.

† Average of 15 men.

ranged from 0.1 to to 6.3  $\mu\text{g./ml.}$  on a dosage schedule of approximately 10 mg./lb. body weight every six hours. The average values for two, four, and six hours after dosage were 2.43, 1.63, and 0.88  $\mu\text{g./ml.}$  respectively, for 33 different samples collected at random in 21 subjects; there was little evidence to suggest cumulative effects from this dosage schedule, since samples of blood collected after two or more doses were not uniformly greater than those seen after initial doses.

The concentrations of oleandomycin in children were considerably in excess of those noted in the comparative pharmacological trials in adults. This difference can be explained by the difference in dosage in relation to body weight (10 mg./lb. in children and 1.5 to 2.0 mg./lb. in adults). The individual results for adults for oleandomycin phosphate, triacetyloleandomycin, and the two erythromycin preparations are shown in table III. The average values (see table IV) for triacetyloleandomycin reflect a clear superiority over oleandomycin phosphate in serum concentrations throughout the first three hours after a single dose of 250 mg.; the peak concentrations were 60 per cent higher for triacetyloleandomycin at two hours after administration. The absorption of triacetyloleandomycin appears to be more prompt than erythromycin, as reflected in the first hour. Peak concentrations of the drug were slightly but not clinically significantly greater in this series of subjects.

In analysis of these data, it becomes apparent that there was considerably more variation in the blood concentrations with erythromycin than with triacetyloleandomycin.

In the urinary studies of the antibiotics, the recovery of triacetyloleandomycin was much greater than for the other preparations studied. The 24 hour excretion was more than twice as much as that for oleandomycin.

Staphylococcal isolates from current infections in outpatients as well as inpatients were selected for sensitivity studies. The results suggested that oleandomycin was an effective antimicrobial agent, probably bactericidal against the strains tested, with an average minimal inhibitory concentration of 1.39  $\mu\text{g./ml.}$  Small numbers of resistant mutants were found in cultures containing oleandomycin in excess of the minimal inhibitory concentration. Only one of the 50 isolates was resistant to greater than 10  $\mu\text{g./ml.}$ , and it was also resistant to erythromycin; the phage pattern was 42D/42E/47/52/52A/53/54. Erythromycin was found to have a minimal inhibitory concentration of 1.15  $\mu\text{g./ml.}$  for 34 strains of these same staphylococci, with 16 of the strains being resistant to greater than 10  $\mu\text{g./ml.}$

There were 16 strains of the epidemic phage pattern (80/81 or 42B/52/81), of which all were inhibited by a concentration of oleandomycin of 2.5  $\mu\text{g./ml.}$  or less, while 14 of these same strains were resistant to erythromycin at concentrations of

10  $\mu\text{g./ml.}$  or greater. There were 5 strains of variants of the epidemic phage pattern. Of these, only one isolate was resistant to erythromycin in concentrations greater than 10  $\mu\text{g./ml.}$

#### DISCUSSION

As a result of these clinical pediatric trials, it is obvious that triacetyloleandomycin is an effective clinical therapeutic agent when gram-positive organisms are the chief bacteriological isolates in disease. Failure to control infection or to improve the patient was noted primarily when an underlying nonbacterial disease was manifest or when gram-negative bacteria (*Aerobacter*, *Pseudomonas*, or *Hemophilus* strains) were the chief isolates.

There were no alternate comparisons made of clinical effectiveness with other agents, but 5 cases responded to the drug when other chemotherapeutic programs had failed.

It was hoped that triacetyloleandomycin might prove to be an effective agent in staphylococcal infections; 12 of 15 patients with diagnoses of osteomyelitis, pneumonia, bronchiolitis, tonsillitis, pyoderma, or abscesses responded favorably. This supports the laboratory data that, at present, it is effective against most staphylococci.

Only 1 strain of 50 coagulase-positive staphylococci was found to be resistant to oleandomycin at this time, but it must be pointed out that neither oleandomycin nor triacetyloleandomycin have been used to any extent for treatment of patients in this institution or in the patients utilizing the outpatient facilities. We have no data or reason to believe that this antibiotic will maintain this high degree of efficiency against staphylococci once it has been used freely, as has been the case with erythromycin; in fact, resistance may be predicted from the *in vitro* studies, in which resistant mutants were found after 18 hours' exposure to oleandomycin in excess of the minimal inhibitory concentration. The drug seems indicated when bacterial resistance and poor clinical responses are occurring with other commonly used antibiotics. Its action has been shown to be both bacteriostatic and bactericidal;<sup>2</sup> after 18 hours' incubation, the sensitivity studies as performed would suggest a bactericidal effect in susceptible organisms.

The pharmacological superiority of triacetyloleandomycin over oleandomycin was corroborated in the adult comparisons. Both serum concentrations and urinary recovery were greater with the former. The serum concentrations were, at all sampling periods, certainly equal to or slightly better than those obtained with tablets of crystalline erythromycin or of erythromycin stearate. Recent experience with the propionyl derivative of erythromycin<sup>1</sup> in this laboratory makes this comparison somewhat less significant, but there can be little question that triacetyloleandomycin was more uniformly absorbed than either of the two erythromycin preparations studied.

Serum concentrations in the children were higher than the adults because of the greater dose. The concentrations obtained would appear, on the basis of *in vitro* tests, to be adequate for successfully treating the majority of staphylococcal isolates in these environs.

#### SUMMARY

1. Triacetyloleandomycin was found to be a clinically effective agent in pediatric infections caused by or associated with gram-positive cocci.

2. A dosage of 10 mg./lb. every six hours produced satisfactory serum concentrations in the children treated as well as effecting clinical improvement or cure.
3. A pharmacological study in adults demonstrated the superiority of triacetyl-oleandomycin over oleandomycin phosphate in producing higher serum concentrations and doubling the urinary recovery.
4. Sensitivity studies with selected pathogenic staphylococci indicate a potential effectiveness against current strains resistant to commonly used antibiotics.

#### BIBLIOGRAPHY

1. FOLTZ, E. L.: A pharmacologic comparison of erythromycin and the propionyl derivative of erythromycin. Unpublished data.
2. HOBBY, G. L., AND LENERT, T. F.: Observations on the mode of action of oleandomycin. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 520.
3. SHUBIN, H.; DUMAS, K., AND SOKMENSUER, A.: Clinical and laboratory studies on a new derivative of oleandomycin. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 679.

# Combination Tetracycline-Oleandomycin Treatment of Acute Respiratory Infection in Childhood

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A combination of tetracycline (67 per cent) and oleandomycin (33 per cent) (Signemycin\*) has been shown to possess marked therapeutic efficacy in many types of infection.<sup>4, 7-11</sup> Critical comment principally based on in vitro and laboratory animal experimentation has not been lacking.<sup>5, 6</sup> To assess the value of this antibiotic combination in treating patients, rather than in laboratory tests, the following investigation was carried out at the Royal Hospital for Sick Children, Glasgow, Scotland.

## MATERIAL AND METHODS

Fifty children with acute infection of the respiratory tract considered to be bacterial in origin were treated with tetracycline-oleandomycin. These cases varied in type from acute tonsillitis to tension pyopneumothorax. The patients ranged in age from 2 weeks to 11 years. In 35 instances (70 per cent), acute respiratory infection complicated underlying systemic disease such as fibrocystic disease (mucoviscidosis), congenital heart disease, acute nephritis, hiatal hernia, toxoplasmosis, primary tuberculosis, and pyloric stenosis. Oral suspension of tetracycline-oleandomycin was given in a dosage of 25 to 41 mg./Kg. body weight per day. The duration of treatment varied from 5 to 21 days but was ordinarily six days.

## RESULTS

The patients fall naturally into two groups, the first consisting of 8 children who had severely toxic conditions with overwhelming infections. This group is summarized in table I.

The fact that two infants with mucoviscidosis died during treatment occasioned no surprise, since they were moribund and had already proved insensitive to a number of antibiotics administered because of resistant staphylococcal bronchopneumonia prior to tetracycline-oleandomycin treatment. Two children died some time after treatment had stopped, 1 a child with mucoviscidosis and recurring staphylococcal bronchopneumonia and the other an infant with congestive heart failure due to congenital heart disease. The remaining 4 patients recovered rapidly and have all remained well.

The second group consists of 42 children with acute respiratory infection varying from acute tonsillitis to bronchopneumonia. Of the 37 patients from whom bacterial culture was taken, 30 per cent yielded a coagulase-positive *Staphylococcus aureus*, 38 per cent yielded a beta-hemolytic *Streptococcus*, and in the remaining 32 per cent the pathogen was undetermined or one of a variety of organisms grown. Rapid recovery followed initiation of tetracycline-oleandomycin treatment in 40 of these 42 children. Persistent vomiting compelled withdrawal of treatment in 1 case, and

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\* The trade name of Chas. Pfizer & Co. for tetracycline-oleandomycin is Signemycin.

TABLE I

*Results in 8 Children with Severely Toxic Conditions and Overwhelming Infections*

Case	Age	Primary disease	Respiratory disease	Results of treatment
1	6 mo.	Mucoviscoidosis	Staphylococcal bronchopneumonia	Died
2	5½ yr.	Mucoviscoidosis	Staphylococcal bronchopneumonia	Improved*
3	7 mo.	Mucoviscoidosis	Staphylococcal bronchopneumonia	Died
4	4 mo.	Congestive heart failure	Bronchopneumonia	Improved*
5	3½ yr.	—	Staphylococcal lung abscess	Well
6	3 mo.	—	Staphylococcal tension pyopneumothorax	Well
7	1⅔ mo.	—	Empyema	Well
8	7 wk.	Prematurity, hypothermia	Bronchopneumonia	Well

\* Died subsequently.

this was followed at once by cessation of vomiting. The remaining patient was quite unresponsive but later proved to be suffering from infectious mononucleosis. The over-all results are shown in table II.

In considering the results as a whole, it might be reasonable to set aside the infants dying of mucoviscoidosis and congestive heart failure as well as the case of infectious mononucleosis. Forty-five children remain, 30 of whom had underlying systemic disease. Forty-four of these responded well to tetracycline-oleandomycin treatment, although 2 were noted to have unpleasant alimentary side effects. Of the total 50 patients, including all with complications, 44 (88 per cent) made a complete recovery apparently related to treatment.

Two cases merit special attention and are summarized briefly.

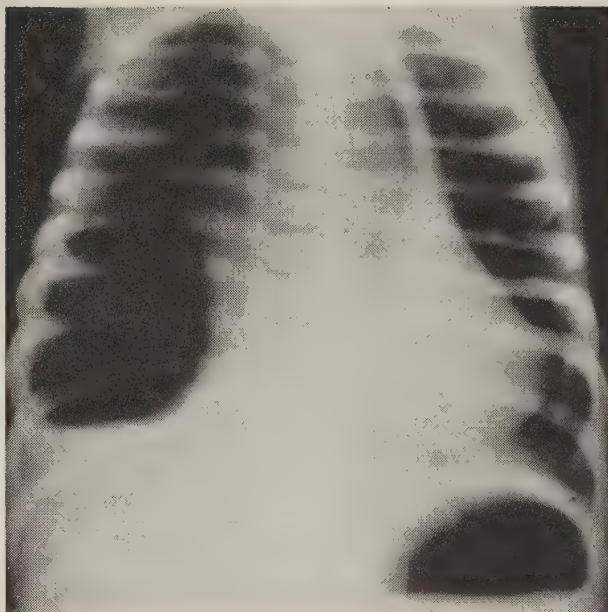
E. H., a 3 month old girl, weighing 4 Kg., had previously received adequate treatment with penicillin, tetracycline, and novobiocin for pneumonia, which had now progressed to a tension pyopneumothorax. She was very dyspneic even in an oxygen tent, the mediastinum was grossly displaced, and she was near to death. The radiographic picture is shown in figure 1. Tetracycline-oleandomycin was given (as a last resort) in a dosage of 40 mg./Kg. body weight per day for eight days. Within 48 hours marked improvement had occurred and this continued steadily despite the advent of diarrhea, which began on the third day of treatment, and oral moniliasis, on the eighth day. The diarrhea persisted for six days, and the combination treatment was then

TABLE II

*Results in 42 Children with Acute Respiratory Infection*

	No.	Comments
Total cases treated	50	
Satisfactory response	46	2 died later, 1 of congestive heart failure, 1 of mucoviscoidosis
Deaths during treatment	2	Both infants with mucoviscoidosis plus staphylococcal pneumonia
Treatment discontinued	2	Alimentary side effects
Misdiagnosis	1	Infectious mononucleosis

FIG. 1. Shown is radiograph of the chest of E. H. prior to tetracycline-oleandomycin therapy.



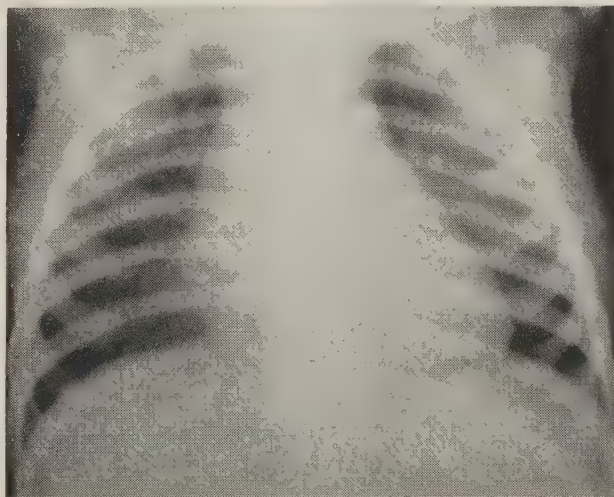
stopped. The monilial infection responded rapidly to gentian violet therapy. Complete recovery ensued and the patient is now well (fig. 2).

J. S., a 3½ year old boy, weighing 15 Kg., had been ill for six weeks. He was feverish, dyspneic, and pale, and had a severe nonproductive cough. Radiography of the chest revealed a huge lung abscess (fig. 3), from which an abundant growth of coagulase-positive *Staph. aureus* was obtained. He had previously received adequate courses of chlortetracycline, chloramphenicol, oxytetracycline, and penicillin with no improvement. Tetracycline-oleandomycin was given in a dosage of 40 mg./Kg. body weight per day for a period of 21 days. The abscess contents continued to yield the same strain of staphylococci, sensitive to tetracycline and oleandomycin, for some 10 days. Combination treatment was continued, the child improved, and complete recovery ensued (fig. 4).

#### DISCUSSION

The advances made in chemotherapy and antibiotic treatment since the turn of the century have brought great benefits to mankind, but inevitably new and complex

FIG. 2. This is a radiograph of the chest of E. H. subsequent to therapy.



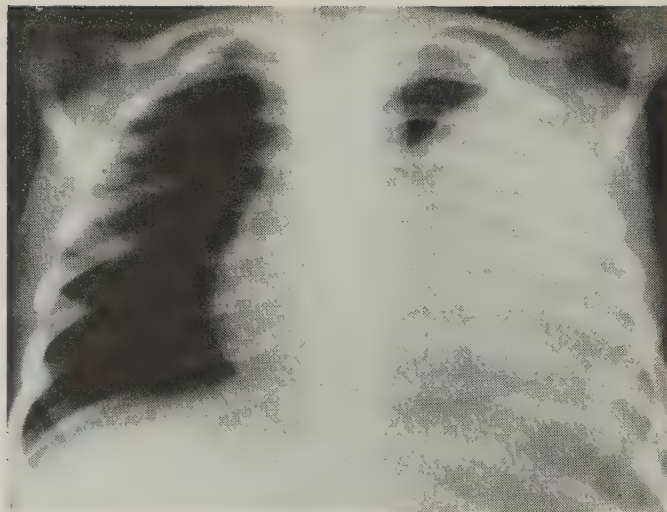


FIG. 3. This is a radiograph of the chest of J. H. prior to tetracycline-oleandomycin therapy.

problems have arisen. One such problem is epitomized by the dichotomy of thought on the administration of antibiotic treatment to the acutely ill patient. To the pure scientist it would appear that the causal organism should be isolated, tested for sensitivity to various antibiotics, and the patient then cured by administering the appropriate antibiotic. This superficially attractive line of reasoning is beset by pitfalls, obvious to the clinician. It is often impossible or impracticable to isolate the causal organism, and several potential pathogens of variable antibiotic sensitivity may be grown. There must be few experienced clinicians who have not found contradictory results between in vitro tests and the treatment of the patient. Correlation between such laboratory tests for antibiotic sensitivity and clinical results is only approximate, and bacteriological findings must be treated with healthy skepticism when clinical results are satisfactory. Furthermore, the testing of such organisms for antibiotic sensitivity is a potentially protracted procedure requiring expert

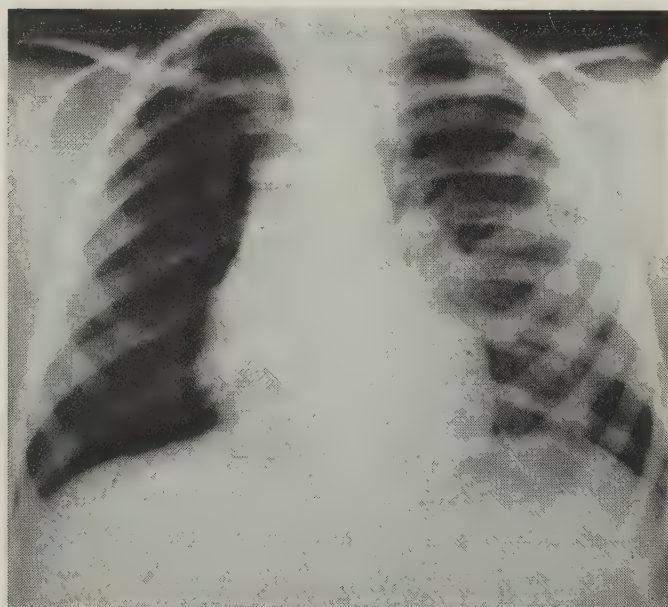


FIG. 4. Shown is radiograph of the chest of J. H. subsequent to therapy.

interpretation. Long delays are intolerable when acute infection demands treatment, and in fact, bacteriological results are usually of retrospective interest.

The clinician, faced with a toxic, febrile patient requiring immediate attention, must therefore use his own judgment to obtain optimal results. Sometimes the likely causal organism can be predicted; thus the patient with acute rheumatism is likely to harbor beta-hemolytic streptococci, lobar pneumonia is probably due to pneumococcal infection, and pyuria probably coliform in origin. Although such crude practical guides are sometimes of assistance, the organism causing acute bacterial respiratory infections is generally indeterminate, but is likely to be a *Staphylococcus*, *Streptococcus*, or pneumococcus. It is where the causal organism is unknown when treatment has to be given that the wide-spectrum antibiotic (mankind's nearest approach to the "panacea") is of great value.

Since no single antibiotic so far found is comprehensive in range, a natural step has been to blend two or more with similar action. Concurrent therapy has three potential advantages: it may widen the range of activity, it may reduce the development of resistant strains where some of the bacteria are naturally resistant to one of the antibiotics,<sup>3</sup> and it may result in synergistic action between the two antibiotics.<sup>2</sup> Against these possible benefits must be placed the risks that more organisms may acquire resistance to both antibiotics than if these had been given separately and that the action of the two antibiotics may be antagonistic. These latter two suggestions are highly speculative as far as human patients are concerned, and it is very doubtful if they can in fact be proved.

For generations it has been accepted that the first essential of treatment is that "it shall do no harm." This aphorism was particularly important before such treatment did much good, but is still true in a relative rather than an absolute sense. It is unfortunate that most potent drugs and antibiotics fall short of the "panacea," not only in range and reliability but also in possessing toxic effects. Such classical examples as arsphenamine, sulfanilamide, penicillin, chloramphenicol, and dihydrostreptomycin spring at once to mind. Sulfamerazine is an excellent example of a substance highly commended on a theoretical basis but dangerous in clinical practice.<sup>1</sup> All broad-spectrum antibiotics produce side effects, and the combination of tetracycline and oleandomycin certainly does not preclude the occurrence of these. In the present series, monilial infection was noted on three occasions. Slight diarrhea occurred in a number of children but distressed only 2; vomiting occurred in 1 patient. No superinfection with staphylococci was observed.

From the results of the present investigation there can be no doubt that tetracycline-oleandomycin is efficacious in a wide range of acute respiratory infections, particularly in severe staphylococcal illness, and such chronic lesions as empyema and lung abscess. That this blend of antibiotics has succeeded where other antibiotics have failed is indisputable, but whether in general it is more effective than other antibiotics is as yet not proved. The incidence of noxious side effects was not remarkable, and it is on clinical results rather than speculative *in vitro* tests that the value of this antibiotic should be assessed.

#### SUMMARY

Fifty children with acute respiratory infections were treated with tetracycline-oleandomycin. Forty-four recovered completely, 2 died, and 2 were improved. The organisms recovered were principally staphylococci and streptococci. Thirty-five children (70 per cent) had severe systemic disease underlying the acute infection.

## BIBLIOGRAPHY

1. ARNEIL, G. C.: Twenty-nine children with sulphonamide haematuria, *Lancet* 1:826, 1958.
2. ENGLISH, A. R.; MCBRIDE, T. J.; VAN HALSEMA, G., AND CARLOZZI, M.: Biological studies on PA 775, a combination of tetracycline and oleandomycin with synergistic activity, *Antib. & Chemo.* 6:511, 1956.
3. FAIRBROTHER, R. W., AND SOUTHALL, J. E.: In-vitro activity of Sigmamycin, *Lancet* 2:974, 1957.
4. FRANK, L., AND STRITZLER, C.: Newer antibiotics in the treatment of acne, *AM & CT* 4:419, 1957.
5. GARROD, L. P.: The erythromycin group of antibiotics, *British M. J.* 2:57, 1957.
6. JONES, W. F., AND FINLAND, M.: Antibiotic combinations, *New England J. Med.* 257:481, 536, 1957.
7. LEVI, W. M., AND KREDEL, F. E.: A clinical trial of Sigmamycin, *J. South Carolina M.A.* 53:178, 1957.
8. OATES, J. K.: Trial of Sigmamycin in non-specific urethritis, *Brit. J. Ven. Dis.* 34:38, 1958.
9. SHUBIN, H.: Clinical evaluation of combined chemotherapy, oleandomycin and tetracycline, *AM & CT* 4:174, 1957.
10. WILLCOX, R. R.: The treatment of non-gonococcal urethritis with tetracycline and oleandomycin in combination, *M. Press* 238:562, 1957.
11. WINTON, S. S., AND CHESROW, E. J.: A clinical study of combined chemotherapy; tetracycline and oleandomycin. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 55.

# The Treatment of Lymphogranuloma Venereum with Triacetyloleandomycin

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During the past three years, we have studied more than 200 cases of lymphogranuloma venereum treated with various antibiotics administered singly or in combination. Among those that we have evaluated in the treatment of this infection are, oxytetracycline, tetracycline, their combinations with oleandomycin, chloramphenicol, erythromycin, and, lately, the triacetyl salt of oleandomycin. Interest in the use of the latter antibiotic for treating lymphogranuloma venereum was stimulated by its effectiveness *in vitro* against some of the large viruses of the family Chlamydiaceae, which is comprised of psittacosis-ornithosis, trachoma, inclusion conjunctivitis, and lymphogranuloma venereum viruses. The agent causing the latter infection—which ranks only below syphilis and gonorrhea as a public health problem—is known currently as *Miyagawanella lymphogranulomatosis*.

We are not aware of any previously published reports on the use of triacetyloleandomycin in the treatment of lymphogranuloma venereum. The present report is concerned with our preliminary knowledge of early lymphogranuloma venereum treated with triacetyloleandomycin. The use of this antibiotic—administered singly—was restricted to the treatment of early cases of lymphogranuloma venereum to determine its effectiveness in a stage of infection in which certain of the previously mentioned antibiotics frequently are quite effectual in causing regression of the lesions. Although some of the lesions of late lymphogranuloma venereum, such as chronic indurated buboes, may respond by regressing under single antibiotic therapy given for extended periods, relapses, nevertheless, may occur after a few months; while lesions, such as elephantiasis, severe esthiomene, and the anorectal syndrome, may not be significantly affected.

Twenty-seven patients, their ages ranging from 23 to 41 years, were treated with triacetyloleandomycin. Twenty-three were men and 4 were women. Sixteen of the men had unilateral lymphadenitis, and 7 had bilateral lymphadenitis of the inguinal region. All of the women had pelvic lymphadenitis, and two also had proctitis. The clinical diagnosis of lymphogranuloma venereum was confirmed by either the intradermal test (Frei), and/or the complement fixation test. The duration of infection in any case probably did not exceed 6 months.

## DOSAGE OF TRIACETYLOLEANDOMYCIN

Since we had no data on the previous use of triacetyloleandomycin in the treatment of lymphogranuloma venereum, we used a discretionary dosage of 1 Gm. per os twice daily for a period of five days.

## CLINICAL RESULTS

Partial regression of the lymphadenitis was generally apparent by the fourth or fifth day of treatment with triacetyloleandomycin, even when there was fluctuation of the glands. Regression of the inflammatory and ulcerous lesions of procti-

tis was noted by the fifth or sixth day of treatment. However, in 11 cases, in which regression of the lymphadenitis was slower than we had expected by the seventh day post-treatment, this dosage of triacetyloleandomycin was repeated. This second course of therapy was uniformly effectual in causing complete regression of the lymphadenitis by the third week post-treatment.

No relapses of either the adenitis or proctitis have been observed during periods of three to eight months post-treatment.

When present, systemic manifestations of early lymphogranuloma venereum, such as fever, headache, malaise, and arthralgia, responded to treatment with triacetyloleandomycin usually by the second day. After discontinuing treatment with oleandomycin, none of these manifestations recurred.

#### TOXIC REACTIONS

No immediate or delayed adverse reactions were observed in these cases as a result of treatment with the above dosages of triacetyloleandomycin.

#### DISCUSSION AND SUMMARY

Although the idiosyncrasies of lymphogranuloma venereum make it difficult to evaluate conclusively the efficacy of a new antibiotic agent in its treatment, it is possible, nevertheless, to generalize by comparison with drugs employed previously in the treatment of similar cases.

Thus, we have gained the impression from treating these 27 cases of early lymphogranuloma venereum with triacetyloleandomycin that: (1) triacetyloleandomycin is a superior antibiotic for the treatment of early lymphogranuloma venereum; (2) triacetyloleandomycin is at least as effectual as the antibiotic combinations in the treatment of early lymphogranuloma venereum, and (3) triacetyloleandomycin is more effective in the treatment of early lymphogranuloma venereum than the previously mentioned single antibiotics when they are given in equivalent dosages.

# The Activity of Phenacridane Chloride Against a Number of Antibiotic-Resistant Staphylococci

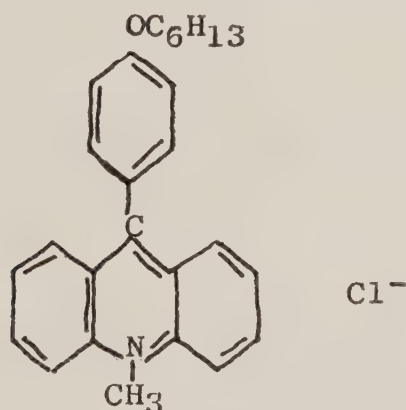
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The problems and implications with regard to antibiotic-resistant staphylococci have become extremely evident during the last several years. The overabundant use of certain antibiotics over the years has probably contributed markedly to the present situation. Numerous clinicians have stated the merits of using antibiotics wisely and judiciously, even reserving some for special needs. Along with this type of thinking has come a re-evaluation of the use of antimicrobial agents other than antibiotics. Particularly in the topical area, the administration of antibiotics is quite often not necessary.

Staphylococci are involved in many of the minor infections of the skin. Therefore, a number of antibiotic-resistant staphylococci have been gathered from several sources. These have been checked for their sensitivity to the usual antibiotics and also for their sensitivity to phenacridane chloride.

Phenacridane chloride has the following structural formula:



The compound is bacteriostatic in extremely high dilution and is bactericidal against both gram-positive and gram-negative organisms.

## MATERIALS AND METHODS

Sensitivity of the strains of *Staphylococcus aureus* was determined with the use of Baltimore Biological Laboratories Sensi-Discs on two-layer brain-heart infusion agar (Difco). Plates were incubated for 24 hours and then the zones of inhibition were observed. Blank discs, the same diameter as the antibiotic discs, were impregnated with 23  $\mu$ g. phenacridane per disc.

The cultures employed in the study were obtained from clinical sources at the institutions listed in table I.

\* The trade name of Johnson & Johnson for 9-para-hexyloxyphenyl-10-methylacridinium chloride (phenacridane chloride) is Micridium.

TABLE I

*Antibiotic Sensitivity of Strains of Staph. aureus*

	Strain of <i>Staph. aureus</i> *†																			
	B 209	B 141	B 210	B 213	B 220	B 225	B 226	B 246	B 253	B 2121	J 419	J 628	J 645	PBB 1	PBB 2	PBB 3	PBB 4	PBB 5	PBB 6	PBB 7
Chloramphenicol	S	S	M	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Erythromycin	S	S	M	S	R	S	S	S	S	S	S	S	S	S	M	S	S	S	S	S
Bacitracin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Chlortetracycline	S	M	M	M	M	M	S	M	M	S	M	M	S	S	S	S	S	M	M	S
Carbomycin	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Dihydrostreptomycin	S	R	M	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	S
Oxytetracycline	S	M	M	M	R	R	R	R	R	M	M	R	R	R	R	M	S	M	M	S
Tetracycline	S	M	M	M	M	M	M	M	M	M	R	M	M	M	S	M	S	S	S	S
Triple sulfonamides	S	M	S	M	S	R	M	M	S	M	S	S	M	S	R	M	S	R	R	M
Neomycin	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Penicillin	S	S	R	R	R	R	R	M	R	R	R	R	R	R	R	R	M	R	R	R
Polymyxin B	S	S	M	M	M	M	R	M	M	M	S	S	M	S	M	S	M	S	M	M
Phenacridane chloride	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

\* B and J strains obtained from G. M. Eisenberg, Philadelphia General Hospital; PBB strains obtained from R. B. Kundsins, Peter Bent Brigham Hospital, Boston.

† S = sensitive; M = moderately sensitive; R = resistant.

## RESULTS

As can be seen in table I, many of the strains tested were resistant to a number of the antibiotics. However, all of the strains tested were uniformly sensitive to phenacridane chloride.

## DISCUSSION

From these results, it is obvious that the acridine derivative is not involved in a cross-resistance with any of the antibiotics. This type of finding has been obtained with certain other synthetic antimicrobial agents.<sup>1</sup>

Because of these results and the other favorable attributes of the compound,<sup>2</sup> it would seem that phenacridane chloride may well be added to the group of non-antibiotic compounds that are becoming the drugs of choice in topical therapy.

## SUMMARY

A number of strains of *Staph. aureus*, isolated clinically, were tested for antibiotic sensitivity and sensitivity to phenacridane chloride. Many of the strains were resistant to a number of the antibiotics but all were uniformly sensitive to the acridine derivative.

## BIBLIOGRAPHY

1. PECK, S. M., AND KANTOR, I.: Germicides versus antibiotics and chemotherapeutics in the control of resistant staphylococcal infections of the skin, *Proc. Sc. Sect. Toilet Goods Assoc.*, no. 29, p. 19, 1958.
2. PRICE, P. B., AND WILSON, B. L.: Acrizane chloride, a promising new skin antiseptic, *Am. Surgeon* 19:792, 1953.

# In Vitro Studies with Leucomycin

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Leucomycin, an antibiotic discovered in Japan, in 1953, by Hata et al,<sup>1</sup> has been reported to be active primarily against gram-positive bacteria, rickettsiae, and large viruses.\* Spirochetes and certain gram-negative bacteria, such as those of the *Neisseria* and *Hemophilus* groups, are also inhibited by leucomycin, while the tubercle bacillus, gram-negative bacilli, and fungi are not.

Although leucomycin is not now commercially available in the United States, dosage forms have been available for some time in Japan. It appears to have rather low toxicity in man. Mikuni et al<sup>2-4</sup> used ointments containing 1 per cent leucomycin (free base or tartrate) to mass-treat school children with trachoma, conjunctivitis, or blepharitis. Numao used leucomycin both orally<sup>5</sup> and intravenously<sup>6</sup> in children and adults. Blood concentrations following the oral administration of 100 to 200 mg. of leucomycin averaged 5.7  $\mu\text{g.}/\text{ml.}$  30 minutes after medication and 1.83  $\mu\text{g.}/\text{ml.}$  four hours after medication. Forty patients with various acute infections (tonsillitis, diphtheria, pertussis, cystitis *Staphylococcus*, abscess, and others) received leucomycin in tablet form in dosages of 50 to 300 mg. given three to eight times daily for 2 to 10 days. Twenty-eight others received comparable doses of leucomycin tartrate oral suspension. Ten minutes after the intravenous injection of 200 mg. of leucomycin tartrate in children, the blood concentrations ranged from 8 to 11.5  $\mu\text{g.}/\text{ml.}$ , and activity was detected in the blood for eight hours. Fifty children treated with intravenous leucomycin received 200 mg. once or twice a day for up to six days. Troches containing 2.0 mg. of leucomycin were also used in some cases.

Matsumae and Onuma,<sup>7</sup> in a study of the effects of leucomycin on several hundred bacterial cultures isolated from patients, found that 98 per cent of 173 *Staphylococcus aureus* cultures were inhibited by leucomycin in concentrations of 2  $\mu\text{g.}/\text{ml.}$  or less. Significantly, 113 of the 173 cultures were resistant to penicillin in concentrations greater than 5 u./ml.

Since there has been an increase in infections due to antibiotic-resistant staphylococci not responding to treatment with certain widely used antibiotics, notably penicillin, there is an active search for new antistaphylococcal agents. Leucomycin may be of promise in this respect.

This paper describes in vitro studies with 247 microorganisms. Forty-seven of these cultures, representing seven genera, were tested for their susceptibility to leucomycin. Since leucomycin resembles erythromycin,<sup>8</sup> the latter antibiotic also was included in the testing. In addition, since previous studies with kanamycin<sup>9</sup> indicated it to be inhibitory for many of these cultures, susceptibility to kanamycin was also included for comparison.

Because leucomycin appears to be very active in inhibiting cultures of *Staph. aureus* resistant to penicillin, 200 *Staph. aureus* cultures were studied for susceptibility to leucomycin, penicillin, and, in most cases, to other antibiotics.

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\* The leucomycin used for the present study is crystalline (1000  $\mu\text{g.}/\text{mg.}$ ) and, of course, considerably more pure than that first produced in Japan.

TABLE I  
Bacteriostatic Activity of Leucomycin, Erythromycin, and Kanamycin  
Against 47 Strains of Organisms Representing 7 Genera

Organism	Number	Minimal inhibitory concentration, $\mu\text{g.}/\text{ml.}$		
		Leucomycin	Erythromycin	Kanamycin
<i>Aerobacter aerogenes</i>	ATCC 8308	500	125	7.8
	ATCC 8329	125	125	3.9
	ATCC 8724	250	250	125
	ATCC 9621	500	125	31.2
	ATCC 884	500	125	15.6
<i>Escherichia coli</i>	ATCC 26	250	62.5	31.2
	ATCC 206	250	125	31.2
	ATCC 133	250	62.5	31.2
	ATCC 4692	500	31.2	31.2
	ATCC 10053	500	250	15.6
<i>Proteus vulgaris</i>	DA 706	250	125	7.8
	DA 711	250	500	15.6
	DA 712	250	250	15.6
	DA 720	250	250	7.8
	DA 723	250	125	3.9
<i>Proteus mirabilis</i>	DA 759	250	500	31.2
	DA 760	500	500	62.5
	DA 764	500	500	31.2
	DA 765	500	500	15.6
<i>Pseudomonas aeruginosa</i>	DA 813	500	250	125
	DA 815	500	125	125
	DA 816	500	125	31.2
<i>Pseudomonas sp.</i>	DA 837	500	125	62.5
	DA 839	125	62.5	
<i>Salmonella typhosa</i>	DA 420B	250	125	3.9
	DA 426	250	125	3.9
	DA 428	250	62.5	7.8
	ATCC 8304	250	62.5	2.0
	ATCC 6539	250	62.5	0.5
<i>Salmonella urbana</i>	DA 917	500	125	7.8
<i>Salmonella typhimurium</i>	DA 918	500	125	31.2
<i>Salmonella paratyphi A</i>	DA 919	250	125	7.8
<i>Salmonella schottmülleri</i>	DA 922	250	125	3.9
<i>Shigella flexneri</i>	DA 953	62.5	31.2	2.0
	DA 969	62.5	31.2	15.6
<i>Shigella sonnei</i>	DA 959	250	31.2	15.6
<i>Shigella paradyserteriae</i>	DA 961	125	62.5	7.8
<i>Streptococcus faecalis</i>	DA 1341	2.0	0.031	—
	ATCC 9790	0.5	0.062	31.2
	ATCC 7080	0.5	0.062	31.2
	ATCC 8022	0.5	0.062	31.2
	ATCC 6569	2.0	1.0	250
<i>Streptococcus viridans</i>	DA 1300	1.0	0.125	62.5
	ATCC 9756	1.0	0.25	500
	ATCC 9758	1.0	0.125	125
	ATCC 7073	0.125	0.031	31.2
	ATCC 9222	0.06	0.015	31.2

#### EXPERIMENTAL

The following genera were represented among the 47 cultures studied for susceptibility to leucomycin, erythromycin, and kanamycin: *Aerobacter*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, and *Streptococcus*. Both clinical isolates and stock cultures were included.

The 200 *Staph. aureus* cultures (pigment-producing, hemolytic, coagulase-positive) were recent clinical isolates. Sixty-seven were isolated from infections and the others were from nasal swabs. The 67 cultures originally isolated from infected

patients were tested for susceptibility to leucomycin and penicillin, and, in many cases, to erythromycin and/or kanamycin. The 133 nasal cultures were all tested for susceptibility to leucomycin, chloramphenicol, erythromycin, novobiocin, oleanomycin, penicillin, streptomycin, and tetracycline, and, in addition, 68 of these were tested for susceptibility to kanamycin.

Leucomycin base with a potency of 1000  $\mu\text{g.}/\text{mg.}$  was used. Since it is quite insoluble in water, it was first dissolved in absolute ethanol to give a concentration of 10,000  $\mu\text{g.}/\text{ml.}$  and then diluted further by slowly adding distilled water to obtain a final concentration of 1000  $\mu\text{g.}/\text{ml.}$  This solution was sterilized by filtration through a sintered glass bacterial filter.

The twofold serial dilution tube technique was used for the susceptibility tests. Trypticase soy broth ( $\text{pH } 7.3$ ) was used to conduct the serial dilution of the antibiotic, to grow the organisms, and to prepare the inoculum. In each case the inoculum consisted of a 1:1000 dilution of a 16 to 18 hour culture of the organism. After incubation of the organism and the serially diluted antibiotic for 16 to 18 hours at  $37^\circ\text{C.}$ , the tubes were inspected for growth. The smallest concentration of antibiotic causing complete inhibition of bacterial growth was recorded as the minimum inhibitory (bacteriostatic) concentration (MIC).

RESULTS

The MIC of leucomycin, erythromycin, and kanamycin for 47 cultures of seven genera are given in table I. Leucomycin and erythromycin were not active against organisms of the genera *Aerobacter*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, and *Shigella*, since the minimum inhibitory concentrations ranged from 62.5 to 500  $\mu\text{g.}/\text{ml.}$  and 31.2 to 500  $\mu\text{g.}/\text{ml.}$ , respectively. The streptococci were inhibited by low concentrations of leucomycin and erythromycin, although in every case less erythromycin was required. Kanamycin was relatively inactive against the *Streptococcus* and *Pseudomonas* cultures, moderately active against *Proteus*, *Salmonella*,

TABLE II  
*Antibiotic Susceptibility Patterns of 200 Cultures of Staphylococcus aureus*

No. of cultures	Sensitive to*	Resistant to*	No. of cultures	Sensitive to*	Resistant to*
19	L P	—	2	L	EP
17	L EP	—	1	L K	EP
5	L EKP	—	2	L CEKNOT	PS
56	L CENOPST	—	2	L CEKNOS	PT
10	L CEKNOPST	—	1	L CENOP	ST
11	L	P	2	L CEKNOP	ST
6	L E	P	2	L CKNOT	EPS
2	L K	P	1	L CKNOS	EPT
1	L CENOST	P	2	L CENO	PST
2	L CEKNOST	P	20	L CEKNO	PST
2	L P	E	1	L CNO	EPST
2	L KP	E	19	L CKNO	EPST
2	L CENOPT	S	2	CKNP	L EOSt
2	L CENOPS	T	2	CKN	L EOPST
4	L CEKNOPS	T			

\* Organisms were judged sensitive if they were inhibited by less than the following quantities: leucomycin (L), 5  $\mu\text{g.}/\text{ml.}$ ; erythromycin (E), 5  $\mu\text{g.}/\text{ml.}$ ; novobiocin (N), 5  $\mu\text{g.}/\text{ml.}$ ; penicillin (P), 5 u./ml.; tetracycline (T), 20  $\mu\text{g.}/\text{ml.}$ ; chloramphenicol (C), 20  $\mu\text{g.}/\text{ml.}$ ; kanamycin (K), 20  $\mu\text{g.}/\text{ml.}$ ; oleanomycin (O), 5  $\mu\text{g.}/\text{ml.}$ ; and streptomycin (S), 20  $\mu\text{g.}/\text{ml.}$

and *Shigella* cultures, and only slightly active against *Aerobacter* and *Escherichia* cultures.

The susceptibility and resistance patterns of the 200 cultures of *Staph. aureus* are shown in table II. It will be noted that 193 cultures were inhibited by leucomycin in quantities of 1  $\mu\text{g.}/\text{ml.}$  or less and three cultures by 2  $\mu\text{g.}/\text{ml.}$  Four cultures were considered leucomycin-resistant, since they were inhibited by 15.6  $\mu\text{g.}/\text{ml.}$  Ninety-three of the 200 cultures were resistant to from one to five of the antibiotics against which they were tested. Eighty (of 200 tested) were penicillin-resistant; 34 (of 178 tested) were erythromycin-resistant; 4 (of 133) were oleandomycin-resistant; 55 (of 133) were streptomycin-resistant; and 58 (of 133) were tetracycline-resistant.

All 78 *Staph. aureus* cultures tested against kanamycin were susceptible to from 0.5 to 7.8  $\mu\text{g.}/\text{ml.}$  The 133 tested against chloramphenicol and novobiocin were all susceptible to from 0.25 to 15.6  $\mu\text{g.}/\text{ml.}$  and 0.06 to 3.9  $\mu\text{g.}/\text{ml.}$ , respectively.

Of the four leucomycin-resistant cultures, two were penicillin-resistant, all were resistant to erythromycin, oleandomycin, streptomycin, and tetracycline, and all were susceptible to chloramphenicol, kanamycin, and novobiocin.

#### COMMENT

There appears to be cross resistance between leucomycin and oleandomycin. Of 200 *Staph. aureus* cultures, only four were leucomycin-resistant. These four were also the only oleandomycin-resistant cultures of 133 tested. Moreover, it seems that in these cases the occurrence of oleandomycin resistance was a predisposing factor in the leucomycin resistance, because the people from whom these cultures were obtained had been exposed to oleandomycin but had had absolutely no contact with leucomycin. While the four cultures were also resistant to erythromycin, a general leucomycin-erythromycin or oleandomycin-erythromycin cross resistance was not in evidence, since 30 other erythromycin-resistant cultures were quite susceptible to leucomycin and oleandomycin. These factors seem to indicate that while leucomycin may be closely related to erythromycin structurally, its mode of action may be more similar to that of oleandomycin, which itself is known to exhibit cross resistance with erythromycin.

#### SUMMARY

In this study the susceptibility to leucomycin and other antibiotics was determined for 247 bacterial cultures of eight genera. Leucomycin was not active against 37 organisms of the genera *Aerobacter*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, or *Shigella*, since the minimum inhibitory concentrations ranged from 62.5 to 500  $\mu\text{g.}/\text{ml.}$  Erythromycin was also inactive against these organisms, exhibiting inhibitory concentrations ranging from 31.2 to 500  $\mu\text{g.}/\text{ml.}$  Leucomycin and erythromycin were very active against the streptococci.

Of 200 *Staphylococcus aureus* cultures tested, 193 were inhibited by leucomycin in concentrations of 1  $\mu\text{g.}/\text{ml.}$  or less and three were inhibited by 2  $\mu\text{g.}/\text{ml.}$  Four *Staph. aureus* cultures were leucomycin-resistant, the minimum inhibitory concentration being 15.6  $\mu\text{g.}/\text{ml.}$  Ninety-three of the 200 cultures were resistant to from one to five antibiotics each, and 80 were penicillin-resistant. Thirty-four of 178 cultures were erythromycin-resistant. Of 133 cultures, four were oleandomycin-resistant, 55 were streptomycin-resistant, and 58 were tetracycline-resistant. All 78

cultures tested against kanamycin were susceptible, as were 133 tested against chloramphenicol and novobiocin.

Cross resistance between oleandomycin and leucomycin appears likely, since four cultures were resistant to both antibiotics, while all other cultures were susceptible to them. It is probable that the cross resistance was of such a nature that the oleandomycin resistance caused the leucomycin resistance. Nevertheless, leucomycin may be of value in combatting infections due to *Staph. aureus* resistant to other antibiotics.

#### ACKNOWLEDGMENT

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#### BIBLIOGRAPHY

1. HATA, T.; SANO, Y.; OHKI, N.; YOKOYAMA, Y.; MATSUMAE, A., AND ITO, S.: Leucomycin, a new antibiotic, *J. Antib. Ser. A.* 6:87-89, April, 1953.
2. MIKUNI, M.; SAGAE, K.; SAITO, S., AND TSUCHIYA, H.: Results of mass treatment of trachoma among school children with leucomycin, *Acta med. et biol.* 4:235-240, Dec., 1956.
3. MIKUNI, M.; SAITO, S.; SAGAE, K., AND TSUCHIYA, H.: Results of mass treatment of trachoma among school children with leucomycin (second report), *Acta med. et biol.* 4:271-275, Dec., 1956.
4. MIKUNI, M.; SAITO, S.; KOBAYASHI, S., AND SAGAE, K.: Effect of leucomycin on conjunctivitis and blepharitis, *Acta med. et biol.* 4:277-280, Dec., 1956.
5. NUMAO, K.: Studies on leucomycin. I. Clinical study. 1. On oral therapy, *J. Antib. Ser. B.* 10:49-55, April, 1957.
6. NUMAO, K.: Studies on leucomycin. I. Clinical studies. 2. Intravenous therapy, *J. Antib. Ser. B.* 10:56-62, April, 1957.
7. MATSUMAE, A., AND ONUMA, M.: Leucomycin sensitivity of bacteria isolated from patients, *J. Antib. Ser. A.* 11:104-108, May, 1958.
8. SANO, Y.; HOSHI, T., AND HATA, T.: Studies on leucomycin. IV. Comparison of leucomycin with erythromycin and carbomycin, *J. Antib. Ser. A.* 7:88-92, May, 1954.
9. WELCH, H.; WRIGHT, W. W.; WEINSTEIN, H. I., AND STAFFA, A. W.: In vitro and pharmacological studies with kanamycin, *Ann. New York Acad. Sc.* 76 (Art. 2): 66-80, 1958.

# Antibiotic and Resistance Studies with Leucomycin

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In 1954, the chemical properties of leucomycin, a product of a *Streptomyces* fermentation, were described by Hata and his co-workers.<sup>1</sup> Independently and at a later date, an antibiotic labelled C-637 was isolated during the course of a screening program conducted jointly by The Wm. S. Merrell Co. and The J. T. Baker Co. By various chemical and microbiological methods leucomycin and C-637 were found to be identical.<sup>2</sup>

Leucomycin is a white, crystalline powder possessing pronounced activity against gram-positive organisms and against *Neisseria* and *Hemophilus*. It is also reported to be active against the spirochetes, rickettsiae, and larger viruses.<sup>3</sup> The bacterial spectrum of this antibiotic resembles those of erythromycin, carbomycin, oleandomycin, and spiramycin. Leucomycin is, however, unique chemically.

## MATERIALS AND METHODS

For our *in vivo* studies, albino mice were used. Microorganisms used in studying leucomycin and/or C-637 were *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Diplococcus pneumoniae* type I, *Staphylococcus aureus* and *Salmonella typhimurium*. Stock cultures were maintained in lyophilized state. Prior to use they were all subcultured in beef infusion broth, with the exception of *Salmonella*, which was grown in Trypticase soy broth. Eighteen hour broth cultures, diluted to give 10 to 100 minimum lethal doses were injected intraperitoneally into 20 Gm. mice. Untreated controls died in 36 to 48 hours following injection with *Streptococcus*, *Klebsiella*, *Diplococcus*, and *Salmonella*. Inoculation with staphylococci produced a more fulminating infection, killing all mice within 24 hours.

## EXPERIMENTAL

*In Vitro Studies.* ANTIBACTERIAL SPECTRUM. For comparative purposes, both C-637 and leucomycin were tested in broth against a variety of microorganisms. From the data listed in table I, it is evident that leucomycin and C-637 possess identical activities.

RESISTANCE STUDIES. The relationship between leucomycin and C-637 was further established by critical resistance studies. Duplicate tubes of broth were inoculated with 0.05 ml. of an 18 hour *Staphylococcus* culture and incubated for two days at 37°C. Tubes were then read for growth, and the culture with the highest concentration of antibiotic showing growth was used as further inoculum. From table II it is evident that staphylococci reach maximum resistance to leucomycin or C-637 within 10 passages.

Two cultures of *Staph. aureus*, one resistant to penicillin and one resistant to penicillin and erythromycin, were used as assay organisms in a plate dilution test. Also included were a penicillin-resistant and a penicillin-sensitive strain of *Bacillus subtilis*. Assays were carried out by the plate dilution method utilizing nutrient agar (Difco) at pH 6.6 for the penicillin assays and streptomycin assay agar

TABLE I  
Bacterial Spectrum of C-637 and Leucomycin In Vitro

Organisms	Minimum inhibitory concentration, $\mu\text{g./ml.}$	
	C-637	Leucomycin
<i>Staphylococcus aureus</i>	0.19–0.37	0.09–0.19
<i>Sarcina lutea</i>	0.09	0.09
<i>Micrococcus lysodeikticus</i>	0.19–0.37	0.19–0.37
<i>Bacillus subtilis</i>	0.37–0.55	0.37–0.55
<i>Bacillus cereus</i>	0.37–0.55	0.37–0.55
<i>Streptococcus</i> sp.	0.55–0.9	0.37–0.55
<i>Staphylococcus aureus</i> , penicillin-resistant	0.19–0.37	0.19–0.37
<i>Mycobacterium tuberculosis</i>	3.7–5.5	3.7–5.5
<i>Mycobacterium smegmatis</i>	3.7–5.5	3.7–5.5
<i>Streptococcus faecalis</i>	0.37–0.55	0.19–0.37

(Difco) at pH 8.0 for the other antibiotics. Phosphate buffer (pH 6.0) was the diluent for the penicillin. All others were dissolved in 50 per cent methanol and further dilutions made in water. From the data in table III, it is evident that leucomycin and C-637 possess activity approaching that of erythromycin. It is also apparent that there is no cross resistance to leucomycin or C-637 by a penicillin-resistant organism. In addition, erythromycin resistance did not influence the activity of leucomycin or C-637.

The next experiment was designed to determine if leucomycin exhibited cross resistance to penicillin or erythromycin. Duplicate tubes of broth containing four-fold dilutions of the respective antibiotics were inoculated with 0.05 ml. of the organism indicated in figure 1. The tubes were read after 48 hours at 37 C.

From figure 1 it is apparent that penicillin resistance does not affect the susceptibility of an organism to leucomycin or C-637. The experiment also served to emphasize that erythromycin resistance does not preclude sensitivity to leucomycin and C-637. Erythromycin resistance did, however, confer a degree of cross re-

TABLE II  
Development of Resistance of *Staphylococci* to Leucomycin and C-637 In Vitro

Antibiotic	Minimum inhibitory concentration ( $\mu\text{g./ml.}$ )											
	1	2	3	4	5	6	7	8	9	10	11	12
Leucomycin	0.2	3.13	6.25	12.5	50	50	50	50	50	100	100	100
C-637	0.4	3.13	6.25	25	50	100	100	200	200	200	200	200

TABLE III  
A Comparison of Antibacterial Activity of Leucomycin, C-637, and Erythromycin

Antibiotic	Minimum inhibitory concentration ( $\mu\text{g./ml.}$ )					
	<i>Staph. aureus</i>	<i>Staph. aureus</i>	<i>Staph. aureus</i>	<i>Staph. aureus</i>	<i>B. subtilis</i>	<i>B. subtilis</i>
Penicillin G*	800	5–650	800	0.03–0.05	800	0.03–0.05
Erythromycin	425–625	0.06–0.08	0.06–0.08	0.02–0.04	0.02–0.04	0.01
Leucomycin	0.2–0.4	0.4–0.6	0.4–0.6	0.4–0.6	0.2–0.4	0.1–0.2
C-637	0.6–0.8	0.6–0.8	0.6–0.8	0.6–0.8	0.4–0.6	0.1–0.2

\* Food and Drug Administration, 1654 units/mg.

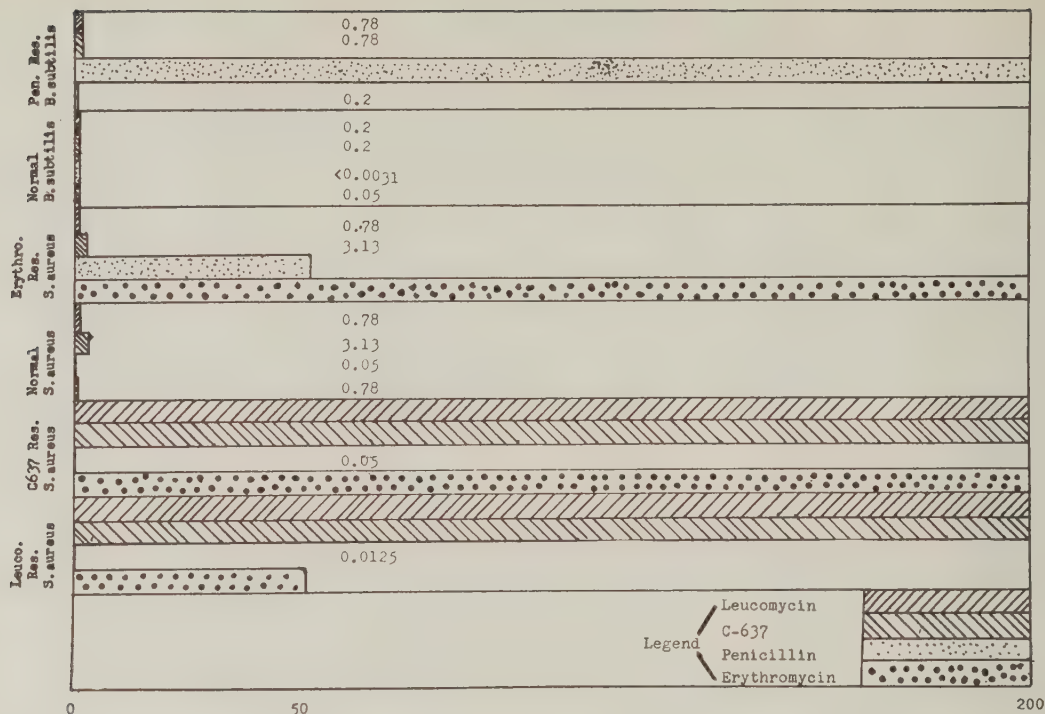


FIG. 1. The activity of leucomycin and C-637 against penicillin-resistant and erythromycin-resistant organisms is shown.

sistance to penicillin. Organisms resistant to leucomycin or C-637 are moderately to definitely resistant to erythromycin.

*In Vivo Studies.* C-637 VERSUS STREPTOCOCCI AND STAPHYLOCOCCI. Mice were infected with 10 to 100 minimum lethal doses of *Str. pyogenes* and *Staph. aureus*, then treated immediately and again six hours later with equal doses of C-637. From the data in table IV, it is evident that C-637 has a pronounced effect on the course of these infections in mice. When given orally, the antibiotic exerted some therapeutic effect, although it was not so striking as that produced by parenteral injection.

COMPARISON OF LEUCOMYCIN AND C-637. Leucomycin and C-637 are essentially the same as determined by chemical analysis and Craig countercurrent distribution.

TABLE IV  
The Intraperitoneal Effect of C-637 on Streptococcal and Micrococcal Infections in Mice

C-637, mg./Kg.	Infecting organism	Ratio, survivors/total
Control	<i>Streptococcus pyogenes</i>	0/12
50	<i>Streptococcus pyogenes</i>	12/12
5	<i>Streptococcus pyogenes</i>	6/6
1.5	<i>Streptococcus pyogenes</i>	1/6
0.5	<i>Streptococcus pyogenes</i>	0/6
Control	<i>Micrococcus pyogenes</i> var. <i>aureus</i>	0/6
50	<i>Micrococcus pyogenes</i> var. <i>aureus</i>	11/12
5	<i>Micrococcus pyogenes</i> var. <i>aureus</i>	8/12
1.5	<i>Micrococcus pyogenes</i> var. <i>aureus</i>	12/12
0.5	<i>Micrococcus pyogenes</i> var. <i>aureus</i>	6/6

TABLE V  
Oral Effectiveness of Leucomycin and C-637 Against a  
*Streptococcal Infection in Mice*

Antibiotic	Total dose for 2 days, mg./Kg.	Ratio, survivors/total
Controls		0/12
Leucomycin	1000	6/6
	300	5/6
	100	3/6
C-637	1000	6/6
	300	6/6
	100	0/6

When tested *in vivo* they exhibit some differences. These differences may primarily be caused by the lack of purity of the C-637.

Both compounds were tested for oral effectiveness against *Str. pyogenes* in mice. The animals were given a dose of either compound and infected six hours after this dose. Immediately after infection the animals received a second dose of antibiotic. Sixteen and 22 hours later they were given the third and fourth doses. From the results listed in table V, it is evident that both substances are absorbed to a certain extent from the intestine and are moderately active at a total dosage level of 300 mg./Kg.

Both C-637 and leucomycin given intraperitoneally were equally effective. The minimum protective dose of leucomycin in this test was 0.15 mg./Kg. and for C-637, 0.5 mg./Kg. As mentioned previously, this difference could be accounted for on the basis of purity of material.

#### SUMMARY

Further studies on leucomycin are presented. In addition, data are tabulated on C-637 and antibiotics possessing similar properties. Both antibiotics exhibit a range of activity similar to that of erythromycin and carbomycin. There is no cross resistance between penicillin-resistant and erythromycin-resistant organisms and leucomycin. Studies are in progress to elucidate further the activities of C-637 and leucomycin. It would appear that leucomycin adds one more effective antibiotic to the armamentarium against such organisms as the resistant *Staphylococcus*.

#### BIBLIOGRAPHY

1. SANO, Y.: Studies on leucomycin. V. Purification and chemical properties, J. Antib. Ser. A 7:93-97, 1954.
2. ANON.: Unpublished data.
3. HATA, T.; SANO, Y.; YOKOYAMA, Y.; ITO, S.; OKAZAKI, S.; TAKANO, K.; ITO H.; OWADA, Y.; SAITO, Y., AND SOEKAWA, M.: Studies on leucomycin. III. Experimental treatment of animals with leucomycin, J. Antib. Ser. A 6:163-171, 1953.

### III. Esters of Low Molecular Weight Aliphatic Acids

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In earlier papers of this series<sup>1,2</sup> the preparation and evaluation of a number of esters of erythromycin were reported. Several more esters were subsequently reported by Clark and Varner.<sup>3</sup> The primary purpose of these previous investigations was the preparation of compounds suitable for use in intramuscular injections or flavored suspensions. The blood levels from oral administration of the esters in these studies were comparable to or less than those obtained with erythromycin.\*

It has now been found that, while esters of erythromycin whose fatty acid portions contain five or more carbon atoms are very poorly absorbed following oral administration, those esters of fatty acids containing two to four carbon atoms give appreciable blood levels. These levels are moderate with the butyrates and reach a peak with the propionate ester.† The acrylate and acetate esters of erythromycin give somewhat lower blood levels than the propionate, but they are still superior to those obtained when erythromycin is administered. A brief summary of representative blood levels following oral administration of 250 mg. capsules is given in table I. The levels reported are for representative groups of 10 patients. More complete data on the levels obtained with erythromycin and erythromycin propionate are given by Griffith.<sup>4</sup>

The physical characteristics of these compounds (tables II and III) indicate that they are the monoacyl esters of erythromycin in which the ester group is formed through the hydroxyl group on the desosamine. A discussion of the basis for this assumption was given by Murphy<sup>1</sup> in the first paper of this series. The structure of erythromycin propionate, based on the original elucidation of the structure of erythromycin given by Wiley et al.,<sup>5</sup> is given in figure 1.

The esters may be prepared by reaction of erythromycin with the appropriate acid halide in a nonreactive solvent, such as anhydrous methanol free acetone under slightly basic conditions. This general method was reported in detail by Murphy.<sup>1</sup> Acid anhydrides may also be used, and a representative preparation of erythromycin propionate from erythromycin and propionic anhydride is given in the experimental portion of this report.

Since several more free hydroxyl groups are available in the erythromycin molecule (see fig. 1), it might be expected that polysubstituted esters could be obtained. However, when erythromycin is reacted with two or more equivalents of an acid chloride, no polyester appears to be produced, but a second monoester as determined by analysis for acyl groups (table II) was isolated. The  $pK_a$  values (table III) obtained on this new monoester indicated that the substitution occurred in the desosamine. It was evident then that the second type of ester, while different from the normal ester, still contained a single ester grouping in the same position. These compounds had very little microbiological activity and a very low activity by ultraviolet method of analysis.<sup>6</sup> The latter and the infrared data, which reveal a

\* The trade name of Eli Lilly & Co. for erythromycin is Ilotycin.

† The trade name of Eli Lilly & Co. for erythromycin propionate is Ilosone.

TABLE I

*Average Clinical Blood Levels Following Oral Administration to Groups of 10 Patients of 250 mg. Capsules of Different Preparations of Erythromycin*

Compound	Hours after administration													
	0		½		1		2		4		6		8	
	A*	B†	A	B	A	B	A	B	A	B	A	B	A	B
Erythromycin	<2		5.2		14.4		21.8		8.8		4.4		<2	
		<0.04		0.10		0.29		0.44		0.18		0.09		<0.04
Erythromycin acetate	<2		7.5		23		48		24		15		9	
		<0.04		0.15		0.45		0.96		0.49		0.30		0.18
Erythromycin acrylate	<2		26		66		44		16		9		4	
		<0.04		0.52		1.31		0.87		0.32		0.17		0.08
Erythromycin propionate	<2		10		47		116		58		22		19	
		<0.04		0.21		0.93		2.32		1.17		0.43		0.38
Erythromycin <i>n</i> -butyrate	<2		5		23		20.5		8.5		2.5		<2	
		<0.04		0.10		0.46		0.41		0.17		0.05		<0.04
Erythromycin isobutyrate	<2		6		17		26.6		19.8		14.8		8.4	
		<0.03		0.09		0.255		0.40		0.30		0.22		0.13
Erythromycin crotonate	<2		<2		<2		<2		<2		<2		<2	
		<0.08		<0.08		<0.08		<0.08		<0.08		<0.08		<0.08
Erythromycin <i>n</i> -valerate	<2		<2		<2		<2		<2		<2		<2	
		<0.04		<0.04		<0.04		<0.04		<0.04		<0.04		<0.04
Erythromycin isovalerate	<2		<2		<2		<2		<2		<2		<2	
		<0.04		<0.04		<0.04		<0.04		<0.04		<0.04		<0.04

\* A, number of tube dilutions.

† B, approximate serum levels,  $\mu\text{g./ml.}$

loss of absorption at  $5.9\mu$  (figs. 2 and 3), indicated that a change in the ketonic group of the erythronolide (macrolide) portion of the molecule had occurred.

A change in the ketonic group was previously reported in which anhydroerythromycin, an internal ketal, is formed from erythromycin in the presence of acid.<sup>5</sup> When the acetate and propionate derivatives of this compound were prepared, they proved to be different from the second erythromycin ester mentioned previously. However, when compounds of the latter type were treated with acid, the corresponding ester of anhydroerythromycin was obtained. In view of this fact, the second type of erythromycin ester may be an intermediate between an erythromycin ester and the ester of its ketal, possibly a stable hemiketal. The relationship among erythromycin, anhydroerythromycin, erythromycin "hemiketal," and their respective esters is illustrated in figure 4. As indicated, the erythromycin "hemiketal" base can be prepared by treatment of erythromycin with glacial acetic acid. Treatment of this product with aqueous hydrochloric acid yields anhydroerythromycin. Comparable conversions can be carried out using an erythromycin ester as a starting material. No satisfactory method for proceeding in the opposite direction has been developed.

The separation and identification of these closely related compounds by paper chromatography can best be accomplished by the use of a completely nonaqueous system. (Developed by Charles Pugh and Harold Bird of the Biochemical Research Division of Eli Lilly & Company). The samples are dissolved in a nonhydroxylated solvent, such as benzene, and the chromatogram developed with a mixture, such as methyl isobutyl ketone (4 parts) and ethyl propyl ketone (1 part). Figures 5 and 6 show the relative mobilities of several representative compounds in this system.

TABLE II  
Physical Characteristics of Different Erythromycin Compounds

Compound	Empirical formula* and molecular weight	Melting point range, C.†	Assay		Carbon, per cent		Hydrogen, per cent		Acyl, per cent	
			Theory	Micro- biological‡	Chemical	Calc.	Found	Calc.	Found	Calc.
Erythromycin acetate	$C_{30}H_{60}NO_{14} \cdot H_2O$ 793.97	127–133	926	905	901	58.99	58.85	9.01	9.14	5.42
Erythromycin propionate	$C_{40}H_{74}NO_{14} \cdot H_2O$ 807.99	122–126	909	928	885	59.46	59.76	9.11	9.04	7.06
Erythromycin acrylate	$C_{40}H_{68}NO_{14} \cdot H_2O$ 805.98	128–132	911	901	872	59.60	59.72	8.88	8.84	
Erythromycin <i>n</i> -butyrate	$C_{41}H_{72}NO_{14} \cdot 2H_2O$ 840.04	134–136	874	819	783	58.45	58.61	9.24	9.09	8.47
Erythromycin isobutyrate	$C_{41}H_{72}NO_{14} \cdot H_2O$ 822.02	119–124	893	905	970	59.90	60.31	9.20	9.17	10.87
Erythromycin crotonate	$C_{41}H_{70}NO_{14} \cdot H_2O$ 820.00	133–137	895	875	719	60.05	59.95	8.97	9.10	
Erythromycin <i>n</i> -valerate	$C_{42}H_{76}NO_{14} \cdot 2H_2O$ 854.07	140–143	860	836	683	59.02	58.92	9.32	9.55	8.69
Erythromycin isovalerate	$C_{42}H_{76}NO_{14} \cdot 2H_2O$ 854.07	142–145	860	812	610	59.02	59.24	9.32	9.21	9.71
Erythromycin hemiketal	$C_{37}H_{67}NO_{13}$ 733.92	134–138		24	0	60.55	60.35	9.20	8.82	
Erythromycin hemiketal acetate	$C_{38}H_{68}NO_{14}$ 775.95	164–168		26	38	60.36	60.20	8.96	9.04	4.81
Erythromycin hemiketal propionate	$C_{40}H_{74}NO_{14}$ 789.98	162–165		25	7	60.81	60.74	9.06	9.12	6.93
Anhydroerythromycin acetate	$C_{38}H_{67}NO_{13}$ 757.93	176–180		2.35	0	61.80	60.97	8.91	9.26	5.44
Anhydroerythromycin propionate	$C_{40}H_{68}NO_{13}$ 771.95	178–181		4.1	0	62.23	61.93	9.01	9.53	7.48

\* Two crystallized forms of erythromycin esters may be prepared. These are the monohydrate and the dihydrate and are distinguishable by means of roentgen-ray diffraction pattern.

† U.S.P. method.

‡ Samples were hydrolyzed for three hours at room temperature in 40 per cent methanol.

TABLE III  
*Electrometric Titration, 66 Per Cent Dimethyl Formamide*

Compound	$pK_a$
Erythromycin	8.6
Erythromycin acetate	6.9
Erythromycin propionate	6.9
Erythromycin acrylate	6.9
Erythromycin <i>n</i> -butyrate	6.8
Erythromycin isobutyrate	6.5
Erythromycin crotonate	6.9
Erythromycin <i>n</i> -valerate	6.9
Erythromycin isovalerate	6.8
Erythromycin hemiketal	8.7
Erythromycin hemiketal acetate	Precipitates in basic form between 6 and 7
Erythromycin hemiketal propionate	Precipitates in basic form between 6 and 7
Anhydroerythromycin	8.3
Anhydroerythromycin acetate	6.8
Anhydroerythromycin propionate	6.8    Some precipitate on the basic side of this $pH$

#### EXPERIMENTAL

*Erythromycin Propionate.* Erythromycin (77.6 Gm.) was dissolved in 300 ml. of anhydrous, methanol-free acetone. Sixteen ml. of propionic anhydride (1.3 equivalents) was added while the solution was stirred at room temperature. After mixing thoroughly, the solution was allowed to stand two hours at room temperature. It was filtered, and 17 ml. of 28 per cent ammonia solution diluted with 400 ml. of water was added. A crystalline precipitate formed very quickly. It was collected by filtration and washed once with 40 per cent aqueous acetone and twice with water. After drying in a vacuum desiccator, the yield was 81 Gm. It was recrystallized by dissolving in acetone and then adding water. No significant change in properties occurred.

The other erythromycin esters listed in table II were prepared by this procedure if the anhydride was available or from the acyl chloride according to the method of Murphy.<sup>1</sup>

*Erythromycin "Hemiketal."* Erythromycin (25 Gm.) was dissolved in 100 ml. of glacial acetic acid and allowed to stand for two hours at room temperature. At the end of this time, the excess acetic acid was removed under reduced pressure by warming on a steam bath. The residue was poured into 300 ml. of saturated sodium bicarbonate solution and additional sodium bicarbonate added, if necessary, to complete the neutralization. The resulting mixture was extracted with two 150 ml. portions of chloroform. The combined chloroform layers were washed with sodium bicarbonate solution and dried over anhydrous sodium sulfate. Removal of the solvent in vacuo gave the product as a white amorphous solid.

*Erythromycin "Hemiketal" Propionate.* FROM ERYTHROMYCIN "HEMIKETAL." Crude erythromycin "hemiketal" (7.7 Gm.) was dissolved in 30 ml. of anhydrous, methanol-free acetone, and 1.6 ml. of propionic anhydride was added with stirring. The resulting substance was allowed to remain at room temperature for two hours. A solution of 1.7 ml. of 28 per cent ammonia solution in 40 ml. of water was then added slowly with stirring to the acetone solution. The resulting precipitate was collected and air dried. It weighed 6.7 Gm. and was recrystallized by dissolving in 30 ml. of acetone and slowly adding 15 ml. of water.

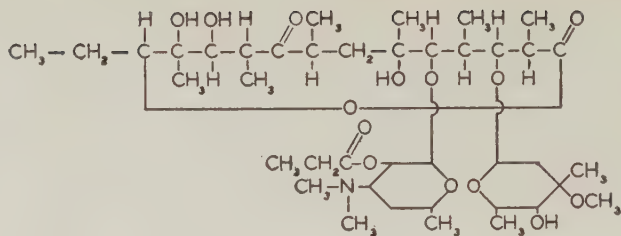


FIG. 1. The structure of erythromycin propionate is illustrated.

**FROM ERYTHROMYCIN PROPIONATE.** Erythromycin propionate (25 Gm.) was dissolved in 100 ml. of propionic acid, boiling point at 139 to 141°C. The solution was allowed to stand for two hours, and the excess propionic acid was removed under reduced pressure while heating on a steam bath. The residue was poured into 300 ml. of saturated sodium bicarbonate solution, and the resulting mixture was extracted with two 150 ml. portions of chloroform. The combined layers were washed with sodium bicarbonate solution, then water, and dried over anhydrous sodium sulfate. After removing the solvent under reduced pressure, the residue was recrystallized by dissolving in 100 ml. of acetone and slowly adding 50 ml. of water. The crystalline precipitate upon drying weighed 20 Gm. By infrared absorption and the roentgen-ray powder diffraction pattern this product was shown to be identical to those from the preceding and following reactions.

**FROM ERYTHROMYCIN.** Erythromycin (31.2 Gm.) was dissolved in 200 ml. of anhydrous, methanol-free acetone, and 15.5 Gm. of sodium bicarbonate was added. While stirring, 8.5 mg. (2.3 equivalents) of propionyl chloride was added dropwise, and stirring was continued at room temperature for one hour thereafter. The mixture was refrigerated overnight, and then 250 ml. of water was added carefully to avoid excessive frothing. The product crystallized as white needles, which were removed by filtration, washed with 30 per cent aqueous acetone, and finally with water. After drying, the product weighed 22 Gm. Further dilution of the filtrate with water gave an oily second crop that was discarded.

**Erythromycin "Hemiketal" Acetate.** Erythromycin "hemiketal" acetate was prepared by acetylation of erythromycin "hemiketal" with acetic anhydride or acetyl chloride or by treatment of erythromycin acetate with glacial acetic acid. No method

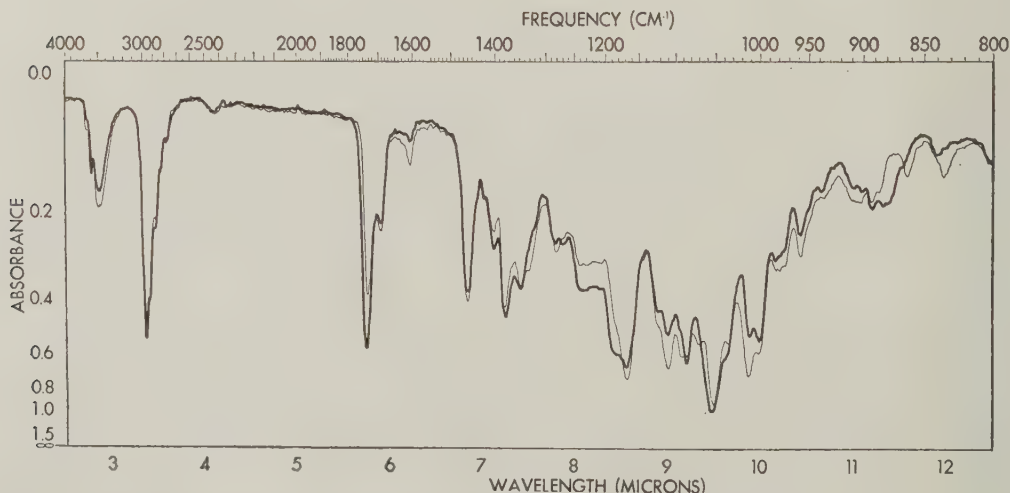


FIG. 2. Infrared spectra for erythromycin propionate, 63 mg./ml. (heavy line), and erythromycin, 62 mg./ml. (thin line), are shown. All infrared spectra were obtained in chloroform solution in 0.095 mm. path on the Perkin Elmer Model 21 spectrophotometer.

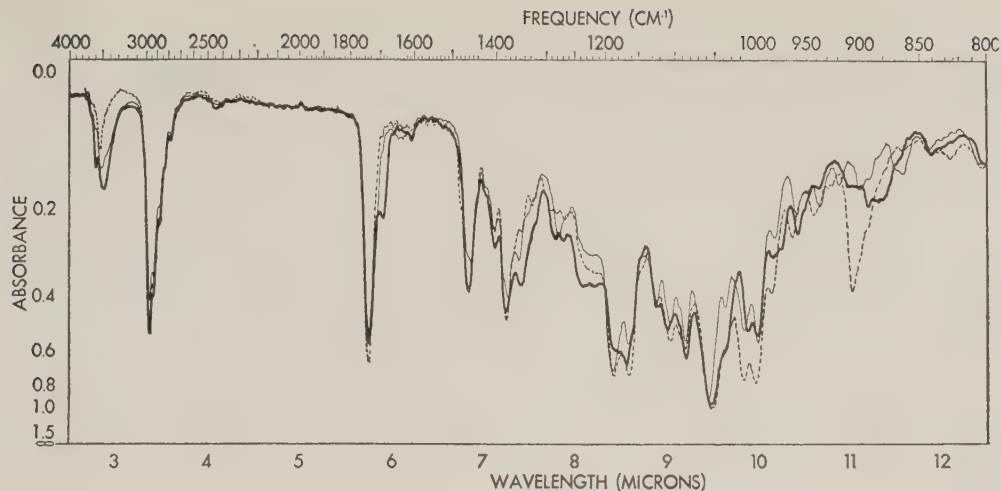


FIG. 3. Infrared absorption spectra are given for erythromycin propionate, 63 mg./ml. (heavy line); erythromycin "hemiketal" propionate, 64 mg./ml. (thin line); and anhydroerythromycin propionate (broken line).

was devised that gave erythromycin "hemiketal" acetate as the predominant product directly from erythromycin. Small quantities were formed and isolated along with erythromycin acetate when 1.5 to 2.3 equivalents of acetyl chloride were used for acetylation of erythromycin.

**Anhydroerythromycin Propionate.** FROM ANHYDROERYTHROMYCIN. Anhydroerythromycin<sup>7</sup> (7.7 Gm.) was dissolved in 30 ml. of anhydrous methanol free acetone and to this was added with stirring 1.6 ml. of propionic anhydride. The resulting solution was allowed to remain at room temperature for two hours. A solution of 1.7 ml. 28 per cent ammonia solution in 40 ml. of water was added to the acetal solution slowly with stirring. The resulting precipitate was collected, dried, and recrystallized by dissolving in 30 ml. of acetone and then slowly adding 20 ml. of water with stirring.

**FROM ERYTHROMYCIN PROPIONATE.** Erythromycin propionate (25 Gm.) was suspended in 500 ml. of water by thorough mechanical stirring. Concentrated hydrochloric acid, diluted with 3 parts of water, was added to reduce the pH to 1.7. Stirring was continued to dissolve the ester, and after the ester had dissolved, the solution pH was again adjusted to 1.7. The solution was allowed to stand at room temperature (23 to 27°C.) for at least 30 minutes with or without agitation. The solution was then neutralized with saturated sodium bicarbonate solution and the resulting mixture extracted with two 150 ml. portions of chloroform. The combined

FIG. 4. The relationship among erythromycin, anhydroerythromycin, erythromycin "hemiketal," and their respective esters is shown.

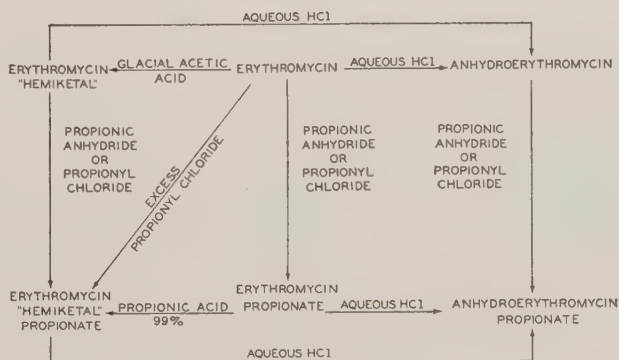




FIG. 5. Illustrated are the paper chromatograms of erythromycin and erythromycin esters. Lane 1, erythromycin; lane 2, erythromycin propionate; lane 3, erythromycin acetate; lane 4, erythromycin acrylate; lane 5, erythromycin *n*-butyrate. The antibiotics are applied in concentrations of 0.05  $\mu\text{g.}/\text{lane}$ .

chloroform extracts were washed with sodium bicarbonate solution and water and dried over anhydrous sodium sulfate. After removal of the solvent under reduced pressure, the residue was recrystallized by dissolving in 100 ml. of acetone and slowly adding 60 ml. of water. Yield was 19 Gm.

FROM ERYTHROMYCIN HEMIKETAL PROPIONATE. Erythromycin hemiketal propionate (3 Gm.) was suspended in 120 ml. of water, and concentrated hydrochloric acid diluted with three parts of water was added until the *pH* became 1.4. The mixture was stirred until all the solid had dissolved and for 30 minutes thereafter. The solution was neutralized with saturated sodium bicarbonate solution and the resulting mixture extracted with chloroform. The chloroform solution was washed with sodium bicarbonate solution and water and then dried over anhydrous sodium

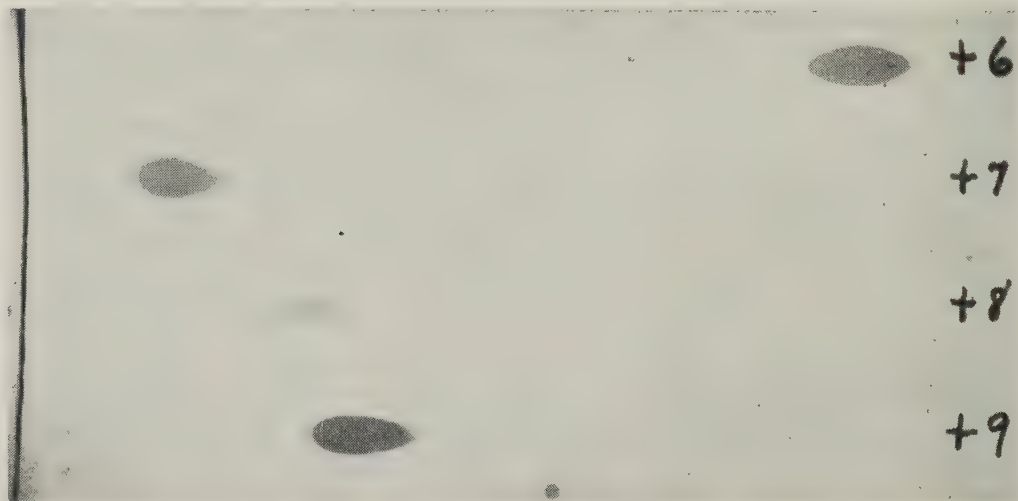


FIG. 6. Additional paper chromatograms are illustrated. Lane 6, erythromycin, 0.05  $\mu\text{g.}$ ; lane 7, erythromycin "hemiketal" propionate, 2.5  $\mu\text{g.}$ ; lane 8, anhydroerythromycin propionate, 60  $\mu\text{g.}$ ; lane 9, erythromycin propionate, 0.05  $\mu\text{g.}$

sulfate. Removal of the solvent left 1.8 Gm. of product, which was recrystallized from 20 ml. of acetone and 10 ml. of water. This product is identical to that isolated from the two preceding methods, as shown by infrared absorption spectra and roentgen-ray diffraction pattern.

#### SUMMARY

Monoesters of erythromycin, with two or three carbon aliphatic acids, when given orally in capsule form gave blood levels superior to those obtained with a comparable dose of the parent antibiotic. The optimum effect was shown with the propionate ester. Further increase in the chain length of the acid leads to much lower blood levels.

A second type of ester derived from erythromycin was shown to be a monoesterified compound of what may be a "hemiketal" of erythromycin, which forms readily by treatment with a weak organic acid. These compounds give a very low chemical assay and have almost no microbiological activity. The monoesterified derivatives of anhydroerythromycin were prepared and also shown to have low activity by chemical and microbiological methods. They were shown to be different from the other two series of esters reported.

#### ACKNOWLEDGMENTS

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#### BIBLIOGRAPHY

1. MURPHY, H. W.: Esters of erythromycin. I. Some water insoluble, substantially tasteless esters. *In: Antibiotics Annual 1953-1954*, New York, Medical Encyclopedia, Inc., 1954, p. 500.
2. STEPHENS, V. C.: Ester of erythromycin. II. Derivatives of cyclic anhydrides. *In: Antibiotics Annual 1953-1954*, New York, Medical Encyclopedia, Inc. 1954, p. 514.
3. CLARK, R. K., JR., AND VARNER, E. L.: New esters of erythromycin, *Antib. & Chemo.* 7:487-489, 1957.
4. GRIFFITH, R. S.: Laboratory and clinical studies with erythromycin propionate. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 364.
5. WILEY, P. F.; GERZON, K.; FLYNN, E. H.; SIGAL, M. V., JR.; WEAVER, O.; QUARCK, W. C.; CHAUVETTE, R. R., AND MONAHAN, R.: Erythromycin. X. Structure of erythromycin, *J. Am. Chem. Soc.* 79:6062, 1957.
6. KUZEL, N. R.; WOODSIDE, J. M.; COMER, J. P., AND KENNEDY, E. E.: Spectrophotometric determination of erythromycin in pharmaceutical products, *Antib. & Chemo.* 4:1234, 1954.

# Pharmacology and Toxicology of Erythromycin Propionate

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Since erythromycin was discovered by McGuire et al<sup>1</sup> of these laboratories, the results of absorption, distribution, excretion, metabolism, and toxicity studies have been reported.<sup>2-11</sup> Clinical experience during the last six years has confirmed the efficacy and low toxicity of this antibiotic. Some esters of erythromycin have been prepared in the past.<sup>12,13</sup> Extensive research on other esters has revealed that certain of them, when administered orally in capsules, give earlier, higher, and more consistent blood levels than those obtained with the base. The propionyl ester<sup>14</sup> has been selected as the one of choice. Results with erythromycin propionate\* reported here show it to be superior to the base in producing better serum concentrations and of the same order of low toxicity.

## MATERIALS AND METHODS

*Acute Toxicity.* For mice and rats, suspensions of appropriate concentrations of erythromycin propionate in 5 per cent acacia were prepared. The materials were triturated until a homogeneous suspension was obtained. One hundred and sixty albino mice, weighing 14 to 18 Gm. after fasting overnight, were given various doses by subcutaneous injection or stomach tube. A total of 60 albino rats, weighing 80 to 120 Gm., were also fasted overnight and administered measured doses in the same manner. Single doses were administered to 11 mongrel dogs in capsules. All animals were observed for one week and the deaths recorded.

*Chronic Toxicity.* Erythromycin propionate was administered to rats by the drug-diet method, as previously described by Anderson et al.<sup>15</sup> A total of 48 albino rats, weighing 80 to 100 Gm., were used. Four groups of 12 each, equally divided as to sex, were fed diets containing 0, 0.05, 0.1, and 0.2 per cent, respectively. Food and water were available ad libitum, and the daily food intake was recorded.

A total of 11 mongrel dogs were given doses twice daily by capsule, including Saturdays and Sundays. Each capsule contained 270 mg. of erythromycin propionate, which is equivalent to 250 mg. of the base. All dogs were observed repeatedly for any side effects. Blood and urine samples were obtained at biweekly intervals for various analyses.

Twenty-four rats and 6 dogs were sacrificed at two months for histological and pathological studies. The remaining animals are still on test.

*Absorption and Excretion.* RATS. One hundred and ninety-eight albino rats, weighing 125 to 150 Gm., were fasted overnight. A single dose of 50 mg./Kg. of erythromycin propionate or the base was administered orally. Groups of rats were decapitated at intervals and the serum from each rat was assayed for antibiotic activity. Additional rats were given other esters, including the acetyl, *n*-butyryl, and isobutyryl, to study the serum concentrations in comparison with those obtained with erythromycin base.

Forty-eight more fasted albino rats, weighing 180 to 200 Gm., were anesthetized under ether. The duodenum was ligated at the junction with the stomach through a medial incision, which was closed with wound clips. After the rats recovered from

\* The trade name of Eli Lilly & Co. for erythromycin propionate is Ilosone.

TABLE I  
*Acute Toxicity of Erythromycin Propionate*

Species	Route of administration	LD <sub>50</sub> , Gm./Kg.
Mouse	Oral	2.87 ± 0.15
	Subcutaneous	>5.0
Rat	Oral	>5.0
	Subcutaneous	>5.0

anesthesia, a dose of 100 mg./Kg. of erythromycin propionate or the base was given orally. Half the rats were decapitated at 30 minutes and the remaining half at 90 minutes. Antibiotic activity in the serum was assayed.

The bile duct of 11 fasted albino rats, weighing 275 to 300 Gm., was cannulated with polyethylene tubing through a medial incision under ether anesthesia. The tubing was brought out through the right posterior abdominal wall and the medial incision was sutured with braided silk. Six rats received a single dose of 100 mg./Kg. of erythromycin base and 5 rats received the propionate. They were placed in Bollman's restrictive cages;<sup>16</sup> food and physiological saline were supplied at all times. The bile was collected in graduated centrifuge tubes at intervals and assayed for antibiotic activity. Urine voided from rats receiving the propionate was saved for paper chromatography studies.

Thirteen more fasted albino rats, weighing 275 to 300 Gm., were anesthetized with 150 mg./Kg. of phenobarbital sodium intraperitoneally. The bile duct was cannulated as described. A dose of 100 mg./Kg. of erythromycin base was administered into the upper part of the duodenum of 6 rats, and the propionate was given to the remaining 7 rats. The bile was collected in graduated centrifuge tubes for two hours and the animals were then decapitated. Antibiotic activity was assayed on the serum and the bile.

Two other fasted albino rats, weighing 300 Gm., were anesthetized with phenobarbital sodium and the thoracic duct was cannulated with polyethylene tubing. The lymph was collected for two hours after an intraduodenal administration of 100 mg./Kg. of the propionate. Both rats were then decapitated and the serum and lymph were assayed for antibiotic activity.

**DOGS.** Four female mongrel dogs with duodenal fistula, weighing between 11.3 and 14.8 Kg., were fasted for 24 hours. Each dog was fed a meal of 200 Gm. of canned dog food one hour before each experiment. A dose of 25 mg./Kg. of erythromycin base or propionate was given orally or intraduodenally through the fistula. Each dog received 30 ml./Kg. of water through a stomach tube immediately after dosing. Blood samples were drawn from the jugular vein at intervals, and urine was collected at two and six hours by catheterization. Erythromycin activity was assayed on the serum and the urine. Six other female dogs without fistula, weighing between 9.2 and 15.6 Kg., were likewise used to study the serum level and urinary excretion of erythromycin after oral administration of erythromycin base and the propionate. A crossover type of experiment was employed in these studies so that the antibiotics were compared against each other in the same dogs. Each animal was allowed to rest for three days or longer before the experiment was repeated.

To study the gastric absorption of this antibiotic, 4 more female fasted dogs were anesthetized with an intravenous injection of 150 mg./Kg. of phenobarbital sodium. An intestinal clamp was placed at the junction of the duodenum and the stomach through a medial incision. Erythromycin base and the propionate were given orally

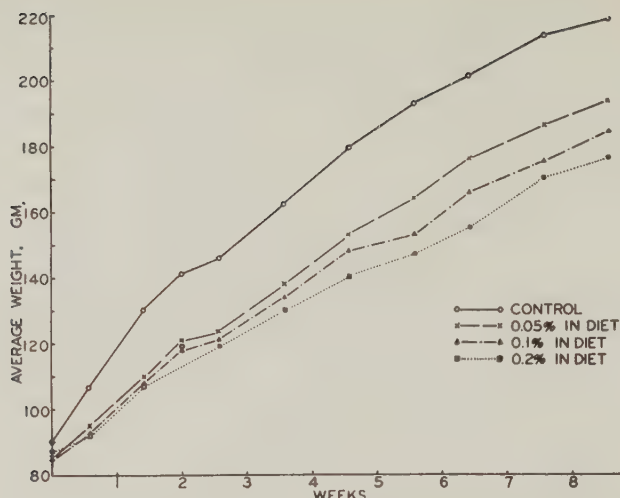


FIG. 1. The growth curves of female rats fed diets containing erythromycin propionate are shown.

to 2 dogs each. Serum concentration and urinary excretion of the antibiotics were determined.

*Paper Chromatography and Bioautography Analysis.* The urine collected from dogs receiving erythromycin propionate orally and the serum, bile, and urine from rats receiving the same antibiotic orally were examined for the presence of this antibiotic by paper chromatography technique in conjunction with bioautography analysis. Two other dogs, weighing 10.2 and 10.9 Kg., respectively, were given 10 mg./Kg. of erythromycin propionate intravenously as a 1 per cent saline suspension. Venous blood samples drawn at five minutes and urine samples collected at one hour were also examined for the presence of this antibiotic.

*Microbiological Assay.* Erythromycin activity was determined in the undiluted serum and lymph and in the urine and bile appropriately diluted with physiological saline by adaptation of the Food and Drug Administration *Sarcina lutea* cup-plate assay.<sup>17</sup> All were compared against a standard sample of erythromycin base. For assays in serum, horse serum was used for setting up the standard curves. Periodically, dog serum was also used, and it was found that horse serum and dog serum gave the same curves. For determinations in urine, bile, and lymph, physiological saline was used.

*Effect on Blood Pressure, Respiration, Intestinal Motility, and the Electrocardiogram.* Mongrel dogs were anesthetized with sodium phenobarbital, 150 mg./Kg.

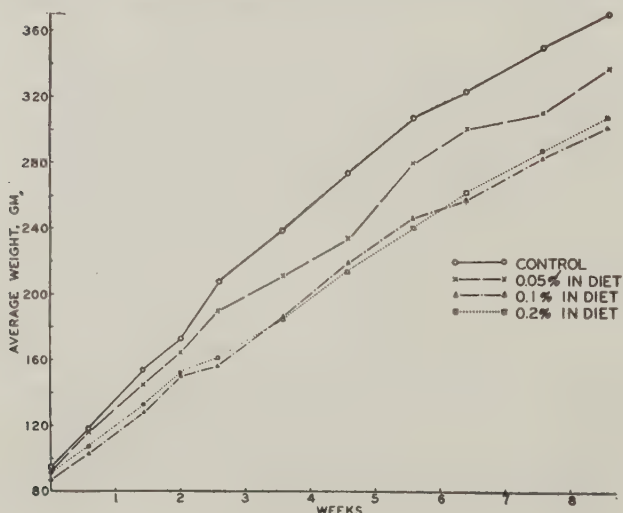


FIG. 2. Growth curves of male rats fed diets containing erythromycin propionate are given.

TABLE II

*Liver Weight (Gm./100 Gm. Body Weight) and Adrenal Weight (mg./100 Gm. Body Weight) of Rats Fed Diets Containing Erythromycin Propionate*

% antibiotic in diet	Sex	Average body weight, Gm.	Average liver weight, wet	Average adrenal weight, wet
0.00	F	218.3	4.16	35.23
0.05	F	193.3	4.00	42.42
0.10	F	184.2	3.49	35.94
0.20	F	176.7	4.18	34.52
0.00	M	370.2	3.98	17.13
0.05	M	338.0	4.47	21.42
0.10	M	302.7	4.20	20.61
0.20	M	308.5	3.78	20.32

by vein. The carotid artery was cannulated and blood pressure recorded with a mercury manometer. The trachea was cannulated and respiration recorded with a tambour through a Haley respirometer. A balloon was placed in the duodenum to record intestinal movements. Electrocardiograms were from the standard lead II and recorded with a Grass polygraph. For intravenous administration, erythromycin propionate was dissolved in polyethylene glycol 200 and injected into the exposed femoral vein. For intraduodenal administration, a suspension containing 25 mg./ml. in 5 per cent acacia was used and given through a tube opening distal to the balloon.

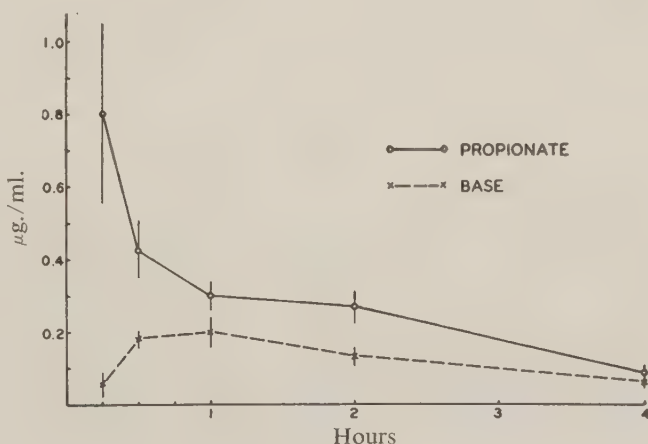
#### RESULTS

*Acute Toxicity.* Erythromycin propionate was found to have a low toxicity. The data for these studies in mice and rats are summarized in table I. Because of the large volume necessary, the  $LD_{50}$  could be determined only for oral administration to mice. After large doses, the mice seemed unaffected for a period of one to two hours. Then a few animals were slightly ataxic. Occasionally, convulsive movements were seen, and death usually occurred within 72 hours. Rats treated orally were observed to have similar symptoms to the mice, although not so pronounced. However, there were no deaths.

Dogs responded to 50 and 100 mg./Kg. of erythromycin propionate given orally by vomiting, thus preventing the actual determination of the amount needed to cause death. At 25 mg./Kg., only 1 of 3 dogs vomited and no vomiting occurred in dogs receiving 10 mg./Kg. The emesis occurred in approximately one hour and ordinarily was not prolonged beyond one or two episodes.

*Chronic Toxicity.* The mean growth curves for the female rats fed various con-

FIG. 3. Serum concentration of erythromycin in rats after oral administration of erythromycin propionate and the base, 50 mg./Kg., is illustrated.



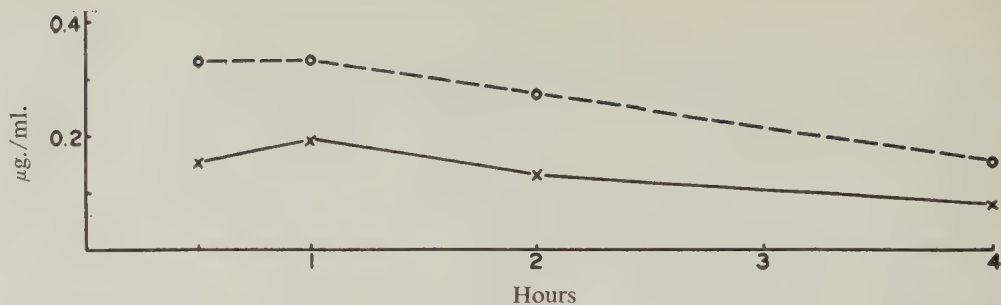


FIG. 4. The serum concentration of erythromycin in rats after oral administration of erythromycin acetate and the base, 50 mg./Kg., is illustrated. x—x, base; o--o, acetyl.

centrations of erythromycin propionate in the diet are found in figure 1 and for the males, in figure 2. From the growth curves, it is apparent that the rats fed 0.05 to 0.2 per cent erythromycin propionate did not grow as rapidly as those fed control diets. The average daily food intake was somewhat less in the groups fed the antibiotic. Since this was evident in the early stage of the study, it appears that the diets were not palatable. After two months, half of the rats in each group were sacrificed and submitted to necropsy. Gross and microscopic examination of the heart, lungs, liver, spleen, kidneys, gastrointestinal tract and thymus, thyroid, pancreas, and adrenal glands revealed no abnormalities. The wet weights of the liver and adrenals were not significantly altered from those of the control rats (table II). Terminal blood counts were within normal ranges.

Eleven mongrel dogs, 6 males and 5 females, received 540 mg. erythromycin propionate in two divided doses each day. After 58 days, 3 males and 3 females were sacrificed and submitted to necropsy. Histological examination was made on the same tissues previously recorded for rats. All tissues were normal in all dogs. Hematocrit and hemoglobin values, erythrocyte, leukocyte, and differential counts remained within normal ranges. Whole blood clotting time and clot retraction time were unchanged in all dogs. The same was true for blood sugar and nonprotein nitrogen values. No glycosuria was noted and only very rarely had any albuminuria been present. Terminal bone marrow counts and sections were normal.

*Absorption and Excretion. SERUM LEVELS.* The serum levels in rats after administration of 50 mg./Kg. of erythromycin base and of various esters are shown in figures 3, 4, and 5. The serum levels of erythromycin produced by the propionate are higher at all times than those obtained with the base. The acetyl ester also gave better serum levels, whereas the *n*-butyryl and isobutyryl esters resulted in lower serum levels than the base. When the rat's stomach was ligated at the junction with the duodenum, oral

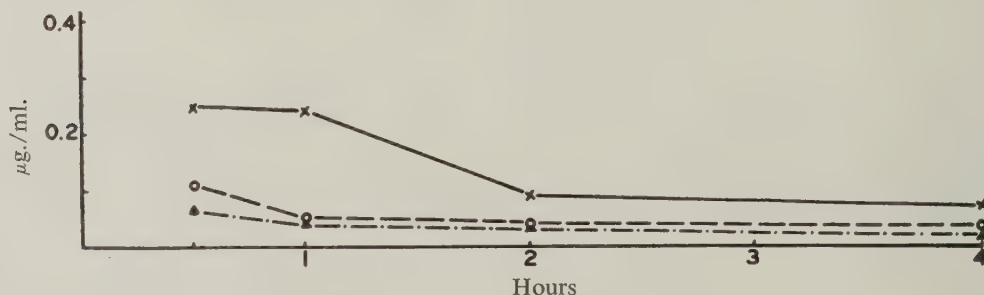


FIG. 5. The serum concentration of erythromycin in rats after the oral administration of erythromycin *n*-butyrate, isobutyrate, and the base, 50 mg./Kg., is shown. x—x, base; o--o, *n*-butyryl; ·—·, isobutyryl.

TABLE III  
*Serum Concentration of Erythromycin in Dogs, 25 mg./Kg.*

Dog	Erythromycin	Route of administration	Time in hours							
			¼	½	1	2	3	4	5	6
Fistula	Base	Oral	<0.03	0.03	0.50	0.41	0.24	0.24	0.18	0.09
		Oral	<0.03	0.03	0.09	0.10	0.07	0.06	0.03	0.03
Fistula	Base	Intraduodenal	0.17	1.34	1.03	0.66	0.38	0.23	0.17	0.11
		Intraduodenal	0.04	0.18	0.22	0.11	0.09	0.05	0.04	0.03
Normal	Base	Oral	<0.03	0.25	0.36	0.26	0.18	0.11	0.11	0.12
		Oral	<0.03	0.03	0.06	0.06	0.05	0.03	0.03	0.04

administration of either the erythromycin base or the propionate resulted in detectable serum concentrations at 30 and 90 minutes in some of the rats.

The average serum levels of erythromycin in 6 normal and 4 fistula dogs are summarized in table III. In contrast to the results with rats, erythromycin base produced higher serum levels at all times than did the propionate after oral administration. Intraduodenal administration of the same dose to the same dogs produced better serum levels than those obtained after oral administration with both antibiotics. Again, erythromycin base gave higher serum concentrations than the propionate.

When erythromycin base was administered orally to each of the 2 dogs whose stomach was clamped off from the duodenum, significant serum concentration was obtained at one hour in 1 dog. It increased and reached a level of 0.48  $\mu\text{g./ml.}$  at the end of five hours. Seven-tenths per cent of the administered dose was excreted in the urine. From the other dog a small amount of erythromycin was also found in the urine. On the other hand, when the propionate was administered to 2 other such operated dogs, no erythromycin activity was detected in the serum and only a very small amount was found in the urine from one dog.

URINARY EXCRETION. The urinary excretion of erythromycin by the normal and fistula dogs is summarized in table IV. After oral administration of erythromycin base, the urinary excretions in six hours averaged 2.25 per cent of the administered dose. Five and twenty-eight hundredths per cent of the dose was excreted in the urine when this antibiotic was given to the duodenum. On the other hand, these dogs excreted much less erythromycin in the urine after oral and intraduodenal administration of the propionate.

Paper chromatography and bioautography analysis of the urine from dogs receiving the propionate failed to demonstrate the presence of this antibiotic. An antibiotic characterized as erythromycin base was found by this technique in these

TABLE IV  
*Urinary Excretion of Erythromycin in Dogs*

Dog	Erythromycin	Route of administration	Per cent of dose		
			0-2 hr.	2-4 hr.	Total
Fistula	Base	Oral	1.18	1.10	2.28
		Oral	0.24	0.24	0.48
Fistula	Base	Intraduodenal	4.18	1.10	5.28
		Intraduodenal	0.35	0.37	0.72
Normal	Base	Oral	1.59	0.66	2.25
		Oral	0.24	0.36	0.60

TABLE V

*Biliary Excretion of Erythromycin in Rats 2 Hours after Intraduodenal Administration*

Erythromycin	Serum concentration, $\mu\text{g./ml.}^*$	Bile volume, ml.	Bile concentration, $\mu\text{g./ml.}$	Per cent of dose	Concentration ratio, bile/serum
Base	0.94 (0.38–1.55)	2.10 (2.0–2.3)	992 (900–1050)	9.17 (7.50–11.67)	1437 (667–2368)
Propionate	1.17 (0.60–1.80)	2.40 (2.2–2.9)	57 (30–90)	0.57 (0.31–0.85)	64 (22–150)

\* Numbers in parentheses are the individual ranges within the groups.

samples. However, both erythromycin propionate and the base were found in the serum and urine of dogs receiving intravenous administration of the propionate.

**BILIARY EXCRETION.** As shown in table V, when erythromycin base was introduced into the duodenum of rats, an average of 9.17 per cent of the dose was excreted in the bile in two hours. The serum concentration of erythromycin in these rats averaged 0.94  $\mu\text{g./ml.}$  The concentration ratios of bile to serum ranged from 667 to 2368. On the other hand, only 0.57 per cent of the dose was recovered when the propionate was given. These rats had an average serum concentration of 1.17  $\mu\text{g./ml.}$  The bile concentrations of erythromycin were only 22 to 150 times those of serum.

The results in table VI show that an average of 1.32 per cent of the administered dose was excreted in the bile of rats in 24 hours after oral administration of the erythromycin base. These rats excreted an average of 0.97 per cent of the dose when the propionate was administered.

By means of paper chromatography and bioautography technique, it was found that erythromycin propionate as well as an antibiotic characterized as erythromycin base occurred in the urine and bile of rats receiving the propionate orally and intraduodenally. To illustrate further the absorption of the propionate from the rat's intestine, a dose of 100 mg. was given intraduodenally to phenobarbital-anesthetized rats weighing 175 Gm. The rats were decapitated at one hour and the serum was analyzed. A large fraction of the microbiological activity in the serum was found to be the administered erythromycin propionate. A small amount of an antibiotic characterized as erythromycin base was also present.

**LYMPH CONCENTRATION.** Nine-tenths and 1.9 ml. of lymph were collected from 2 rats in two hours after intraduodenal administration of erythromycin propionate. The lymph concentrations of erythromycin from these 2 rats were 0.70 and 0.95  $\mu\text{g./ml.}$  and those of the serum were 1.00 and 1.15  $\mu\text{g./mg.}$ , respectively.

**Pharmacodynamics.** Two dogs received by rapid intravenous injection 5, 10, and 20 mg./Kg. of erythromycin propionate. Equivalent volumes of the solvent, polyethylene glycol 200, were administered in alternate doses. Doses of 10 and 20 mg./

TABLE VI

*Biliary Excretion of Erythromycin in Rats after Oral Administration*

Erythromycin	Per cent of dose			
	0–1 hr.*	1–7 hr.	7–24 hr.	Total
Base	0.36 (0.05–0.70)	0.78 (0.32–1.34)	0.18 (0.10–0.31)	1.32 (0.54–2.14)
Propionate	0.11 (0.03–0.34)	0.57 (0.41–0.84)	0.29 (0.07–0.56)	0.97 (0.63–1.74)

\* Numbers in parentheses are the individual ranges within the groups.

Kg. were followed by transient falls in mean arterial pressure varying from 14 to 40 mm. of mercury. The solvent also produced transient depressor responses of 12 to 28 mm. of mercury. The net fall due to the antibiotic was 6 mm. of mercury after 10 mg./Kg. and 28 mm. of mercury after 20 mg./Kg.

Transient increases in respiratory rate were noted after injection of both erythromycin propionate and the solvent. However, after 20 mg./Kg. of the drug, the increase in rate was more marked and of longer duration than that after the solvent.

All doses of erythromycin propionate were followed by an increase in duodenal activity. The increase was slight after 5 mg./Kg. but marked after 10 and 20 mg./Kg.

The heart rate was not altered by doses of 5 mg./Kg. In one dog, doses of 10 and 20 mg./Kg. produced slowing of the heart rate, and the electrocardiogram, which had a negative T wave in the control period, changed to an isoelectric T wave. In the second dog there was a transient decrease in heart rate, but no change in the electrocardiogram, after 20 mg./Kg.

Four dogs received doses of 25 mg./Kg. by tube into the duodenum. The blood pressure change ranged from a decrease of 8 to 34.5 mm. of mercury over a period of 30 to 90 minutes. There were no significant changes in respiration. In 3 dogs there was an increase in duodenal motility. The configurations of the electrocardiograms were not altered, but 2 of the animals showed a slight increase in heart rate.

Approximately 2½ hours after this dose, 50 mg./Kg. was given via the intestinal tube. This dose was followed by a fall in mean arterial pressure in 3 of the 4 dogs. The falls ranged from 10 to 20 mm. of mercury occurring 60 to 100 minutes after administration. The control pressure of the fourth dog was low, and 75 minutes after 50 mg./Kg., there was an increase in blood pressure of 44 mm. of mercury. All the animals showed an increase in intestinal motility, more marked than after 25 mg./Kg. Two of the dogs had an increase in heart rate, and in 1 animal the T wave of the electrocardiogram was inverted.

#### DISCUSSION

Chronic administration of erythromycin propionate in the diet of rats for two months at concentrations equivalent to 50 to 200 mg./Kg./day produced some retardation of growth but no visceral or hematopoietic damage. The wet weights of liver and adrenals were not significantly altered. It is believed that the decreased food intake was a result of altered palatability and was accountable for the retardation of growth.

Dogs tolerated doses of erythromycin propionate equivalent to 40 to 50 mg./Kg. daily for eight weeks without evidence of toxicity. Ten of 11 dogs gained weight and showed no evidence of abnormality. No visceral damage was evident in the 6 dogs that were sacrificed at the end of two months. There was no evidence of any significant hematopoietic change in any dog.

In the rat, the presence of the erythromycin propionate in the urine, bile, and serum after oral and intraduodenal administration was demonstrated by paper chromatography and bioautography analysis. This indicates its absorption as such from the gastrointestinal tract. The occurrence of erythromycin propionate in the urine of human subjects after oral administration has also been reported.<sup>14</sup>

The propionate was also hydrolyzed to some extent in the body of rats, liberating erythromycin, which was found in the urine, bile, and serum. These studies, however, do not show whether this hydrolysis took place in the gastrointestinal tract,

or whether the propionate was absorbed as such from the tract and then hydrolyzed, or whether both possibilities existed.

No propionate was found in the urine and serum samples of dogs when it was administered orally and intraduodenally. It appears that the propionate has to be hydrolyzed in the gastrointestinal tract before the hydrolyzed product, erythromycin, is absorbed in this species. However, both erythromycin propionate and an antibiotic characterized as the base appeared in the serum and urine of dogs when erythromycin propionate was administered intravenously. This indicates that this antibiotic is hydrolyzed to some extent when it gets into the circulation.

Both erythromycin base and propionate are slightly absorbed from the ligated stomach of the rat. However, the major site of absorption of both is in the intestines, as indicated by the larger biliary recoveries of both antibiotics after intraduodenal administration (table V) than after oral administration (table VI). This confirms our previous observation<sup>6</sup> that erythromycin base and several derivatives are chiefly absorbed from the intestines in the rat.

In dogs, both erythromycin base and propionate produced higher serum levels at all times after intraduodenal administration than after oral dosage (table III), indicating that both are mainly absorbed in the dog's intestines. This was reported previously with erythromycin base and erythromycin B.<sup>18</sup> As demonstrated in these studies, no serum concentration was detected and no significant amount of activity was found in the urine when the propionate was placed in the stomach, which was clamped off from the duodenum, although the erythromycin base was absorbed slightly through the dog's stomach. Apparently, hydrolysis of erythromycin propionate takes place in the intestine of dogs, where the erythromycin is absorbed.

Erythromycin propionate has been reported to produce better serum concentration in human subjects.<sup>14</sup> This has been demonstrated also in rats (fig. 3 and table V). The superiority of erythromycin propionate to the base in producing higher serum concentrations may be due to its slow and limited excretion through the bile. Rats receiving erythromycin base excreted more antibiotic in the bile than those receiving the propionate (tables V and VI). Erythromycin base was reported to be eliminated in large amounts in the bile of rats<sup>4,6</sup> and of dogs.<sup>4</sup> As demonstrated in the present studies, dogs failed to absorb the propionate as such. This apparently contributes to its failure in producing better serum concentrations.

Large doses (20 mg./Kg.) of erythromycin propionate administered intravenously or intraduodenally to anesthetized dogs produced a slight and transient fall in blood pressure, increase in respiratory rate, and stimulation of duodenal motility. This dose did not result in any significant change of the electrocardiogram. The dosages used in these experiments were 8 to 15 times the therapeutic dosage for man. Similar changes have been found after 20 to 40 mg./Kg. of erythromycin base intravenously in anesthetized dogs.

#### SUMMARY

1. In rats, oral administration of erythromycin propionate produced higher serum concentrations than those obtained with the erythromycin base. Among the other esters studied, erythromycin acetate also resulted in higher serum levels.

2. Both erythromycin propionate and the base appeared in the blood, urine, and bile of rats after oral administration of the propionate. On the other hand, only erythromycin base was found in the blood and urine of dogs receiving erythromycin propionate orally.

3. Rats excreted the antibiotic in the bile to a greater degree after administration of erythromycin base than after administration of the propionate.
4. Both the erythromycin base and the propionate were chiefly absorbed from the intestines of the rat and dog.
5. Acute toxicity studies in mice, rats, and dogs showed that erythromycin propionate had the same order of low toxicity as erythromycin base.
6. Chronic toxicity studies in rats at concentrations equivalent to 50 to 200 mg./Kg./day in the diet revealed some retardation of growth after two months but no visceral or hematopoietic damage. Dogs with daily doses of more than 50 mg./Kg. for eight weeks failed to show any pathological changes.
7. Large doses of the propionate both by intravenous and intraduodenal administration were followed by a slight and transient decrease in blood pressure, increase in respiratory rate, and stimulation of intestinal motility. The propionate did not cause any significant change in the electrocardiogram.

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#### BIBLIOGRAPHY

1. MCGUIRE, J. M.; BUNCH, R. L.; ANDERSON, R. C.; BOAZ, H. E.; FLYNN, E. H.; POWELL, H. M., AND SMITH, J. W.: "Ilotycin," a new antibiotic, *Antib. & Chemo.* 2:281, 1952.
2. ANDERSON, R. C.; HARRIS, P. N., AND CHEN, K. K.: The toxicity and distribution of 'Ilotycin,' *J. Am. Pharm. A.* 41:555, 1952.
3. LEE, C.-C.; ANDERSON, R. C., AND CHEN, K. K.: Tissue distribution of erythromycin in rats, *Antib. & Chemo.* 3:920, 1953.
4. LEE, C.-C.; ANDERSON, R. C., AND CHEN, K. K.: The biliary and urinary excretion of erythromycin in dogs. *In: Antibiotics Annual 1953-1954*, Medical Encyclopedia, Inc., New York, 1954, p. 485.
5. LEE, C.-C.; ANDERSON, R. C.; BIRD, H. L., JR., AND CHEN, K. K.: Reabsorption of erythromycin and a microbiologically active metabolite in the bile of dogs and rats. *In: Antibiotics Annual 1953-1954*, Medical Encyclopedia, Inc., New York, 1954, p. 493.
6. LEE, C.-C.; ANDERSON, R. C., AND CHEN, K. K.: Site of absorption of erythromycin in rats, *Antib. & Chemo.* 4:926, 1954.
7. LEE, C.-C.; ANDERSON, R. C., AND CHEN, K. K.: Renal clearance of erythromycin, *Proc. Soc. Exper. Biol. & Med.* 88:584, 1955.
8. ANDERSON, R. C.; HARRIS, P. N., AND CHEN, K. K.: Further toxicological studies with 'Ilotycin' (erythromycin, Lilly), *J. Am. Pharm. A.* 44:199, 1955.
9. LEE, C.-C.; ANDERSON, R. C., AND CHEN, K. K.: Distribution and excretion of radioactivity in rats receiving N-methyl-C<sup>14</sup> erythromycin, *J. Pharmacol. & Exper. Therap.* 117:265, 1956.
10. LEE, C.-C.; ANDERSON, R. C., AND CHEN, K. K.: The excretory products of N-methyl-C<sup>14</sup> erythromycin in rats, *J. Pharmacol. & Exper. Therap.* 117:274, 1956.
11. LEE, C.-C.; HARRIS, P. N.; ANDERSON, R. C., AND CHEN, K. K.: Absence of thyroid changes by erythromycin in chickens, *Antib. & Chemo.* 7:132, 1957.
12. MURPHY, H. W.: Esters of erythromycin. I. Some water-insoluble, substantially tasteless esters. *In: Antibiotics Annual 1953-1954*, Medical Encyclopedia, Inc., New York, 1954, p. 500.
13. STEPHENS, V. C.: Esters of Erythromycin. II. Derivatives of cyclic anhydrides. *In: Antibiotics Annual 1953-1954*, Medical Encyclopedia, Inc., New York, 1954, p. 514.
14. GRIFFITH, R. S.; STEPHENS, V. C.; WOLFE, R. N.; BONIECE, W. S., AND LEE, C.-C.: Preliminary studies on propionyl erythromycin, *AM & CT* 5:609, 1958.
15. ANDERSON, R. C.; HENDERSON, F. G., AND CHEN, K. K.: The rat as a suitable animal for the study of prolonged medication, *J. Am. Pharm. A.* 32:204, 1943.
16. BOLLMAN, J. L.: A cage which limits the activity of rats, *J. Lab. & Clin. Med.* 33:1348, 1948.
17. KIRSHBAUM, A.; BOWMAN, F. W.; WINTERMERE, D. M., AND FRIEDMAN, E. R.: A cup-plate method for the determination of erythromycin concentrations in serum and other body fluids, *Antib. & Chemo.* 3:537, 1953.
18. LEE, C.-C.: Duodenal fistula dog as test animal for acid-stable or labile compounds, *Proc. Soc. Exper. Biol. & Med.* 94:547, 1957.

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In 1952, McGuire et al<sup>1</sup> reported finding a new antibiotic produced by an actinomycete, *Streptococcus erythreus*, that was effective against large viruses, most gram-positive bacteria, several gram-negative bacilli, rickettsia, spirochetes, and a few protozoa. Since the introduction of this new antimicrobial agent, erythromycin,\* numerous clinical reports establishing its clinical effectiveness have appeared in the literature, including several excellent reviews.<sup>2-11</sup>

Murphy,<sup>12</sup> Stephens,<sup>13</sup> and Wiley et al<sup>15</sup> have previously reported the preparation of certain esters of erythromycin. Other esters have been prepared by Stephens and Conine.<sup>14</sup> Of the various esters prepared, studies indicate that the propionyl ester of erythromycin produces clinical blood levels that are superior to any other form of erythromycin used for comparison.<sup>14</sup>

Preliminary studies with propionyl erythromycin† in the laboratory and the infectious disease service at Indianapolis General Hospital have been completed.<sup>16</sup>

## METHODS

*In Vitro Activity.* Representative strains of bacteria were tested for sensitivity to erythromycin base and propionyl erythromycin. Each antibiotic was diluted in brain-heart infusion broth using the twofold serial dilution method. The minimal inhibiting concentration was determined by noting the tube with no growth containing the least amount of antibiotic.

*Erythromycin Blood Levels.* To compare propionyl erythromycin with capsules of erythromycin tablets protected by an acid-resistant coating, serum samples were obtained in fasting, ambulatory, healthy adult subjects following a single 250 mg. oral dose.

Blood was drawn before and at ½, 1, 2, 4, 6, and 8 hours after administration of each of three preparations. The sera were assayed using a modified Rammelkamp twofold serial dilution method.

One hundred subjects received research or pilot lots of propionyl erythromycin, 50 the coated tablets of erythromycin base, and 20 individuals were given erythromycin base in capsules.

*Multiple Dose Study.* Ten subjects were given both propionyl erythromycin and erythromycin base in tablets protected by an acid-resistant coating in a dosage of 250 mg. every six hours. Five subjects received each medication for three days and the other 5 individuals received each medication for five days. Blood samples were drawn each day before the morning dose and at 1, 2, 4, and 6 hours after administration. Urine samples were collected from 5 subjects at six hour intervals for four days and assayed with the serial dilution method. Saliva samples were collected from 2 subjects at two hour intervals for six days on the third day of medication. Assays after filtering were made using the same twofold serial dilution technique.

\* The trade name of Eli Lilly & Co. for erythromycin is Ilotycin.

† The trade name of Eli Lilly & Co. for propionyl erythromycin is Ilosone.

TABLE I

Sensitivity of Microorganisms to Erythromycin and Erythromycin Propionate in Broth Dilution Tests\*

Test organism	Minimum inhibitory concentration, μg./ml.	
	Erythromycin	Erythromycin propionate
<i>Staphylococcus aureus</i> 209P	.2	.2
<i>Staphylococcus aureus</i> H408 (penicillin- and tetracycline-resistant)	.39	.39
<i>Staphylococcus aureus</i> H435 (penicillin-resistant)	.39	.39
<i>Staphylococcus albus</i> H241A (novobiocin-resistant)	.78	.78
<i>Diplococcus pneumoniae</i> †	.025	.025
<i>Streptococcus pyogenes</i> C203†	.0125	.0125
<i>Klebsiella pneumoniae</i>	3.13	3.13
<i>Shigella paradysenteriae</i>	6.25	6.25
<i>Brucella bronchiseptica</i>	3.13	3.13
<i>Vibrio metschnikovii</i>	.39	.2
<i>Proteus vulgaris</i>	100.0	100.0
<i>Escherichia coli</i>	50.0	25.0
<i>Pseudomonas aeruginosa</i>	50.0	50.0
<i>Aerobacter aerogenes</i>	100.0	50.0
<i>Alkaligenes faecalis</i>	50.0	50.0

\* Brain-heart infusion broth (Difco) was used as the test medium.

† Three per cent blood was added to the medium when testing these organisms.

Two crossover studies were used to compare erythromycin with triacetyloleandomycin. In the first series, 10 subjects received both coated tablets of erythromycin base and triacetyloleandomycin. In the second series, an additional 10 subjects were given both propionyl erythromycin and commercial capsules of triacetyloleandomycin with glucosamine. A single 250 mg. dose was used for each preparation. Techniques of administration, sampling, and assay were the same as those described previously.

Patients from Indianapolis General Hospital requiring antibiotic therapy were treated with propionyl erythromycin. A dosage of 250 mg. or 500 mg. every six hours was given, the higher dose for the more severe infections. Isolation of the causative organism was made when possible, and follow-up cultures were obtained in those patients who showed no improvement or clinical evidence of failure.

Twelve patients with various types of chronic infections, such as infected decubiti and varicose ulcers, who had been receiving antibiotic therapy, were given pro-

FIG. 1. Times serum was diluted before inhibition was lost are indicated, using a 250 mg. dose as a base, tube dilution method, beta-hemolytic *Streptococcus* C203. \*—\*, propionyl erythromycin capsules, 100 subjects; x-----x, erythromycin base tablets with coating, 50 subjects; o—•—o, erythromycin base capsules, 20 subjects.

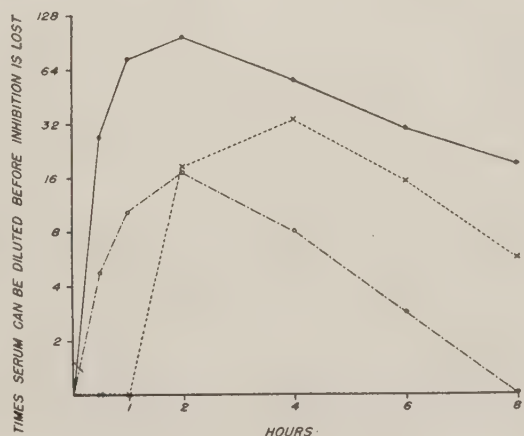


TABLE II  
Comparison of Antibiotic Activity

Preparation	Hours							Subjects
	0	½	1	2	4	6	8	
Comparison in micrograms*								
Propionyl erythromycin	0	0.54	1.39	1.92	1.06	0.53	0.33	100
Tablet erythromycin base	0	0	0	0.36	0.54	0.23	0.09	50
Capsule erythromycin base	0	0.1	0.22	0.34	0.15	0.06	0	20
Comparison as reciprocal†								
Propionyl erythromycin	0	27	80	108	57	30	20	100
Tablet erythromycin base	0	0	0	19	33	15	6	50
Capsule erythromycin base	0	5	11	17	8	3	0	20

\* Fasting subjects were given a single dose of 250 mg.

† Expressed as reciprocal of maximum inhibiting dilution of subject's serum (number of times serum can be diluted before losing its inhibition of a pathogenic strain of beta-hemolytic *Streptococcus*).

propionyl erythromycin, 500 mg. every six hours. Hemograms, urinalyses, blood urea nitrogen, transaminase, bilirubin, and thymol turbidity were obtained at twice weekly intervals. These data were compared for evidence of toxicity.

## RESULTS

Comparison of activity of erythromycin base and propionyl erythromycin in vitro indicates their spectra to be identical. Potency against the organisms within the spectrum is the same for the ester as for the base (table I).<sup>17</sup>

Preliminary clinical blood level studies, using research and pilot lots of propionyl ester of erythromycin in fasting normal, ambulatory subjects, showed an earlier onset of therapeutic concentration and higher and more prolonged high concentrations than with either erythromycin base in capsules or erythromycin base in tablets protected by an acid-resistant coating (fig. 1).

Table II contains the results obtained in subjects receiving the research and pilot lots of propionyl erythromycin and erythromycin base as tablets and capsules. The results have been expressed both in micrograms and in the number of times the subject's serum can be diluted before losing antibiotic activity against a pathogenic strain of beta-hemolytic *Streptococcus*. This was measured using a modified Rammekamp twofold serial dilution technique with *Streptococcus* C203 as the indicator organism. It is apparent that at one half hour the propionyl ester has higher levels than the other two preparations. The peak concentration achieved by the propionyl erythromycin is approximately four times that of erythromycin in coated tablets and seven times greater than the levels reached by the capsules of erythromycin base. The duration of the ester is also greater than the other two forms.

Multiple dosage studies verify the finding of early high levels of propionyl erythromycin when compared to the levels obtained with coated tablets of erythromycin base. There is no evidence of cumulation with a dosage regimen of 250 mg. every six hours (tables III and IV).

Following the dosage of propionyl erythromycin, the antibiotic activity in the urine is approximately twice that obtained during the period of erythromycin administration (table V).

TABLE III

Comparison of Serum Levels Propionyl Erythromycin with Coated Tablets Erythromycin Base Following Multiple Doses, 250 mg. Every 6 Hours for 3 Days

Subject no.	Blank	Hour after dose													
		First day						Second day						Third day	
		1	2	4	6	0*	1	2	4	6	0*	1	2	4	6
Propionyl Erythromycin (6-30-58 to 7-2-58)															
1	<0.04	5.12	2.56	1.28	0.64	0.64	1.28	5.12	2.56	1.28	0.64	0.64	2.56	5.12	2.56
2	<0.04	0.64	0.64	0.32	0.32	0.96	2.56	1.28	0.64	0.64	0.32	2.56	2.56	1.28	0.64
3	<0.04	0.16	1.28	0.32	0.08	0.16	2.56	2.56	0.64	0.64	0.16	0.32	0.64	0.32	0.08
4	<0.04	2.56	2.56	1.28	0.64	1.28	2.56	2.56	2.56	1.28	0.32	0.32	1.92	0.64	0.32
5	<0.04	0.64	1.28	1.28	0.32	1.28	1.92	2.56	0.64	0.32	0.32	1.28	2.56	1.92	2.56
Average	<0.04	1.82	1.66	0.89	0.40	0.86	2.17	2.88	1.40	0.83	0.35	1.02	2.04	1.85	1.23
Erythromycin Base (7-8-58 to 7-10-58)															
1	<0.04	<0.04	<0.04	<0.04	<0.04	1.28	1.28	1.28	1.92	0.96	0.08	0.24	2.56	2.56	1.28
2	<0.04	<0.04	<0.04	<0.04	0.16	0.16	0.64	0.64	1.28	0.64	0.16	0.16	0.32	0.64	0.16
3	<0.04	<0.04	0.32	0.32	0.16	0.16	0.16	0.16	0.48	0.16	0.64	0.16	0.16	0.64	0.16
4	<0.04	<0.04	0.16	0.64	0.48	0.64	0.64	0.96	1.28	0.64	0.64	1.28	1.28	1.28	0.64
5	<0.04	<0.04	0.64	0.64	0.24	0.32	0.16	0.64	0.64	0.32	0.16	0.16	1.28	0.64	0.32
Average	<0.04	<0.04	0.22	0.32	0.20	0.51	0.57	0.73	1.12	0.54	0.33	0.40	1.12	1.15	0.51

\* Sample taken immediately before morning dose.

TABLE IV

*Comparison of Propionyl Erythromycin with Coated Tablets Erythromycin*

		Hours after administration												
Subject no.	Date	First day					Date	Second day					Date	0*
		Blank	1	2	4	6		0*	1	2	4	6		
Propionyl erythromycin														
6	7-28-58	<0.04	0.04	0.64	1.28	0.64	7-29-58	0.48	0.64	2.56	2.56	3.84	7-30-58	0.64
7		<0.04	2.56	1.92	0.96	0.32		0.32	0.32	2.56	1.28	0.64		0.64
8		<0.04	<0.04	3.84	1.92	0.96		5.12	10.24	7.68	5.12	2.56		1.28
9		<0.04	1.28	1.92	0.48	0.24		0.32	0.64	1.28	0.64	0.24		0.64
10		<0.04	<0.04	0.32	0.64	0.16		0.24	0.48	1.28	0.64	0.32		0.16
Av.		<0.04	0.77	1.72	1.05	0.46		1.29	2.46	3.07	2.04	1.52		0.67
Erythromycin base														
6	8-4-58	<0.04	<0.04	0.06	0.08	0.04	8-5-58	0.32	0.16	0.16	0.96	0.24	8-6-58	0.12
7		<0.04	<0.04	0.48	0.32	0.08		0.08	0.08	0.64	0.96	0.32		0.32
8		<0.04	<0.04	2.56	1.28	0.32		1.92	1.92	1.92	2.56	0.64		0.32
9		<0.04	0.16	0.24	0.16	0.08		0.32	0.32	0.64	1.28	0.64		0.64
10		<0.04	<0.04	0.96	0.32	0.08		0.24	0.16	1.28	0.64	0.16		0.16
Av.		<0.04	<0.04	0.86	0.43	0.12		0.57	0.52	0.92	1.28	0.40		0.31

\* Sample was taken immediately before morning dose.

Filtered saliva, which was collected in 2 subjects over a six hour period on the third day of each multiple dosage study, did not show assayable quantities of the base or propionyl ester.

Comparative studies between erythromycin and triacetyloleandomycin demonstrated greater antibacterial activity against beta-hemolytic *Streptococcus* C203 with the two forms of erythromycin studied. The average subject's serum following propionyl erythromycin can be diluted without losing antibiotic activity 20 times more than it can after taking capsules of triacetyloleandomycin with glucosamine (table VI). Acid-resistant tablets of erythromycin base also show higher serum concentrations than triacetyloleandomycin (table VII).

Twenty-three patients were referred to the infectious disease research service for therapy with erythromycin (table VIII). They were given propionyl erythromycin, 250 to 500 mg. every six hours. The higher dosage was used in the more severe infections. Recovery was uneventful in most instances. Surgical drainage was performed where needed, making evaluation of antibiotic therapy difficult.

Spectacular results were seen in 2 patients with severe infections. One was a staphylococcal cellulitis of the face. Temperature and white cell count were elevated, and the face was painful and very tender over the swollen areas. Both eyes were swollen shut. The patient was hospitalized, and after two 500 mg. doses of propionyl erythromycin, the pain and tenderness were markedly reduced, the eyes were open enough to see, and the patient was hungry. Recovery was uneventful.

The second case was a potential breast abscess with fever and swollen, hot, and exquisitely tender breast. This patient was treated in the outpatient department and received 500 mg. every six hours. The swollen, tender lymph nodes in the axilla were the first to disappear, then the breast became less tender, and finally the abscess resolved without need of drainage.

Those patients receiving propionyl erythromycin, 500 mg. every six hours for two weeks to one month, for evaluation of side effects and effect on the blood, liver, and kidney, were found to have no significant alteration in hemograms, urinalyses,

TABLE IV  
Base Following Multiple Doses, 250 mg. Every 6 Hours for 5 Days

Hours after administration																	
Third day					Fourth day					Fifth day							
1	2	4	6	Date	0*	1	2	4	6	Date	0*	1	2	4	6	8	
2.56	2.56	2.56	1.28	7-31-58	3.84	2.56	1.92	5.12	1.28	8-1-58	0.48	0.32	0.64	1.28	0.64	0.24	
1.28	2.56	1.28	0.64		0.32	2.56	1.28	1.92	0.96		0.32	2.56	1.28	1.28	0.64	0.32	
5.12	5.12	2.56	1.92		3.84	2.56	5.12	3.84	2.56		0.32	5.12	2.56	1.28	0.64	0.48	
1.28	2.56	1.28	0.64		0.96	2.56	2.56	1.28	0.64		0.64	0.64	0.64	0.64	0.24	0.16	
1.28	1.28	0.96	0.64		0.48	1.28	1.92	1.28	0.64		0.16	0.24	1.28	1.28	0.32	0.24	
2.30	2.81	1.72	1.02		1.88	2.30	2.56	2.68	1.21		0.38	1.77	1.28	1.15	0.49	0.28	
0.64	0.32	0.64	0.96	8-7-58	0.08	0.08	0.16	0.32	0.24	8-8-58	0.32	0.32	0.24	0.32	0.16	0.16	
0.32	1.28	0.64	0.32		0.32	0.32	1.28	0.64	0.32		0.16	0.16	0.64	0.64	0.32	0.16	
0.32	0.32	1.28	0.64		1.92	<0.04	5.12	2.56	0.96		0.16	0.16	0.32	0.32	0.16	0.08	
0.64	1.28	1.28	0.64		0.64	0.64	1.28	1.28	0.64		0.32	0.48	0.64	0.64	0.32	0.16	
0.16	0.96	0.48	0.16		0.04	1.28	0.64	0.32	0.16		0.16	0.16	0.64	0.32	0.16	0.08	
0.41	0.83	0.86	0.54		0.60	0.46	1.69	1.02	0.46		0.22	0.25	0.49	0.38	0.22	0.12	

blood urea nitrogen, transaminase, bilirubin, or thymol turbidity after therapy was completed, compared with tests performed before starting the medication.

DISCUSSION

The alteration of the molecule by the addition of the propionyl radical apparently does not change the spectrum or potency of erythromycin.

If dilutions are made on the basis of erythromycin content in the molecule of propionyl erythromycin, the minimal concentration required to inhibit representative strains of bacteria is identical to that concentration necessary for erythromycin to prevent multiplication of the test organism.

Animal studies do not suggest a difference in absorption between the propionyl

TABLE V  
Urine Excretion Levels in Subjects\* Receiving Multiple Doses (250 mg. Every 6 Hours) of Propionyl Erythromycin and Coated Tablets Erythromycin Base

Date	Hr. after dose, mg./ml.				24 hours		Vol. total, ml.
	6	12	18	24	μg./ml.	mg. total	
Propionyl erythromycin							
7-28-58	2.67	4.48	3.17	2.52	13.27	12.84	983
7-29-58	6.34	8.41	11.47	10.19	41.0	36.43	938
7-30-58	12.18	6.32	4.27	6.68	46.0	29.46	769
7-31-58	9.60	4.48	3.33	11.40	26.5	28.83	1043
Av., 24 hr.	7.89	5.82	5.56	7.69	31.67	26.91	835
Erythromycin base							
8-4-58	2.54	1.6	.90	2.8	11.3	7.96	709
8-5-58	6.21	4.27	2.04	5.5	23.9	18.05	752
8-6-58	8.29	3.13	2.38	4.1	20.4	17.99	920
8-7-58	14.12	2.94	1.7	1.9	24.24	20.64	938
Av., 24 hr.	7.78	2.98	1.75	3.5	20.0	16.10	839

\* Same subjects as reported in table IV.

TABLE VI

Comparison of Propionyl Erythromycin with Triacetylleandomycin-Glucosamine, Single Dose, 250 mg.

Subject	Date	Tube dilution, † hr. after dose						Hr. after dose, µg./ml.							
		Blank	1/2	1	2	4	6	8	Blank	1/2	1	2	4	6	8
J. D.	6-18-58	<2	<2	16	16	8	4	<2	<.04	<.04	.32	.32	.16	.08	<.04
P*	6-23-58	<2	<2	4	4	4	<2		<.4	<.4	.8	.8	.8	<.4	<.4
T†															
J. L.	6-18-58	<2	64	128	128	64	32	32	<.04	1.28	2.56	2.56	1.28	.64	.64
P	6-23-58	<2	<2	2	2	2	2	2	<.4	<.4	<.4	.4	.4	.4	<.4
T															
R. W.	6-18-58	<2	<2	<2	16	16	8	4	<.04	<.04	<.04	.32	.32	.16	.08
P	6-23-58	<2	<2	2	4	4	2	4	<.4	<.4	.4	.8	.8	.4	.8
T															
R. H.	6-18-58	<2	<2	128	256	64	32	16	<.04	<.04	2.56	5.12	1.28	.64	.32
P	6-23-58	<2	<2	<2	4	2	2	<2	<.4	<.4	<.4	.8	.4	.4	<.4
T															
C. J.	6-18-58	<2	<2	<2	8	4	2	2	<.04	<.04	<.04	.16	.08	.04	.04
P	6-23-58	<2	<2	<2	<2	<2	<2	2	<.4	<.4	<.4	<.4	<.4	<.4	<.4
T															
R. W.	6-18-58	<2	16	128	128	128	64	32	<.04	.32	2.56	2.56	2.56	1.28	.64
P	6-23-58	<2	—	16	16	8	4	4	<.4	<.4	.8	1.6	.8	.4	.4
T															
J. W.	6-18-58	<2	4	8	64	32	16	16	<.04	.08	.16	1.28	.64	.32	.32
P	6-23-58	<2	—	16	16	8	4	2	<.4	—	3.2	3.2	1.6	.8	.4
T															
C. R.	6-18-58	<2	8	16	32	32	16	16	<.04	.16	.32	.64	.64	.32	.32
P	6-23-58	<2	<2	<2	<2	<2	<2	<2	<.4	<.4	<.4	<.4	<.4	<.4	<.4
T															
L. S.	6-18-58	<2	16	64	64	64	16	16	<.04	.32	1.28	1.28	1.28	.32	.32
P	6-23-58	<2	<2	<2	4	4	<2	<2	<.4	<.4	.8	.8	.8	.4	.4
T															
C. McB.	6-18-58	<2	2	32	128	64	64	32	<.04	.04	.64	2.56	1.28	1.28	.64
P	6-23-58	<2	<2	<2	4	4	2	2	<.4	<.4	<.4	.8	.8	.4	.4
T															
Av.	P	<2	11	52	84	47.6	25.4	16.4	<.04	.22	1.04	1.68	.95	.51	.33
T		<2	<2	3	4.6	3.2	1.4	1.2	<.4	<.4	.6	.92	.64	.28	.24

\* P = propionyl erythromycin. † T = triacetylleandomycin with glucosamine.

‡ Number of times serum can be diluted before minimal inhibition is lost, using beta-hemolytic *Streptococcus* as the indicator.

TABLE VII  
Comparison of Erythromycin Base in Coated Tablets with Triacetylleandomycin, Single Dose, 250 mg.

Subject	Date	Tube dilution, $\frac{1}{2}$ hr. after dose						Hr. after dose, $\mu\text{g./ml.}$							
		Blank	$\frac{1}{2}$	1	2	4	6	8	Blank	$\frac{1}{2}$	1	2	4	6	8
J. D.	6-13-58	<2	<2	<2	32	16	8	4	<.06	<.06	<.06	.96	.48	.24	.12
I* C†	6-11-58	<2	<2	4	8	4	2	2	<.4	<.4	.8	1.6	.8	.4	.4
J. L.	6-13-58	<2	<2	4	64	32	16	4	<.06	<.06	.12	1.92	.96	.48	.12
I C	6-11-58	<2	<2	<2	<2	2	2	<2	<.4	<.4	<.4	<.4	.4	.4	<.4
R. W.	6-13-58	<2	<2	2	8	16	8	14	<.03	<.03	.03	.12	.24	.12	.06
I C	6-11-58	<2	<2	2	4	4	2	2	<.4	<.4	<.4	.8	.8	.4	.4
R. H.	6-13-58	<2	<2	<2	<2	32	16	8	<.03	<.03	<.03	<.03	.48	.24	.12
I C	6-11-58	<2	<2	<2	4	4	2	2	<.4	<.4	<.4	.8	.8	.4	.4
C. J.	6-13-58	<2	<2	<2	<2	16	8	4	<.03	<.03	<.03	<.03	.24	.12	.06
I C	6-11-58	<2	<2	<2	<2	<2	2	2	<.4	<.4	<.4	.8	.4	.4	<.4
J. P.	6-13-58	<2	<2	<2	<2	32	32	16	<.03	<.03	<.03	.03	.48	.48	.24
I C	6-11-58	<2	<2	2	8	4	4	2	<.4	<.4	<.4	1.60	.8	.8	.4
S. H.	6-13-58	<2	<2	<2	<2	<2	<2	<2	<.03	<.03	<.03	<.03	<.03	<.03	<.03
I C	6-11-58	<2	<2	4	4	2	2	2	<.4	<.4	.8	.8	.4	.4	<.4
G. B.	6-13-58	<2	<2	<2	32	64	32	8	<.06	<.06	<.06	.96	1.92	.96	.24
I C	6-11-58	<2	<2	<2	4	2	<2	<2	<.4	<.4	<.4	.8	.4	<.4	<.4
J. S.	6-13-58	<2	<2	<2	2	32	16	4	<.06	<.06	<.06	.06	.96	.48	.12
I C	6-11-58	<2	<2	<2	<2	<2	<2	<2	<.4	<.4	<.4	<.4	<.4	<.4	<.4
C. D.	6-13-58	<2	<2	<2	64	32	8	4	<.06	<.06	<.06	1.92	.96	.24	.12
I C	6-11-58	<2	<2	<2	4	2	2	2	<.4	<.4	<.4	.8	.4	.4	<.4
Av.	I C	<2	<2	<2	20	27	14	6	<.05	<.05	<.05	.60	.67	.34	.12
	C	<2	<2	<2	4	3	2	<2	<.4	<.4	<.4	.8	.6	.5	<.4

\* I = erythromycin base in coated tablet. † C = triacetylleandomycin.

‡ Number of times serum can be diluted before minimal inhibition is lost, using beta-hemolytic *Streptococcus* as the indicator organism.

TABLE VIII  
*Clinical Results Following Propionyl Erythromycin Therapy*

Subject	Diagnosis	Bacteriology	Dosage, mg. every 6 hr.	Duration, days	Results	Side effects
Mild Infections						
V. P.	Acute pharyngitis	Nonspecific	250	4	Improved	Aftertaste
R. G.	Acute pharyngitis	<i>H. influenzae</i>	500	4	No improvement	Soft stools
H. B.	Acute gingivitis		250	4	Improved	
T. R.	Furunculosis	<i>Staph. aureus</i>	250	5	Improved	
J. W.	Chronic acne	<i>Staph. aureus</i>	500	Contin.	No new lesions	
R. R.	Paronychia		250	3	Healed	
R. C.	Sinusitis		500	5	Improved	
F. K.	Diabetic, cellulitis, foot	<i>Staph. aureus</i> and <i>Proteus</i>	250	7	Healed	
R. C.	Acute sinusitis		500	3	Improved	Nausea
S. C.	Cellulitis, foot		250	5	Healed	Pruritus ani
M. V.	Chronic hydradenitis	<i>Staph. aureus</i> and <i>Proteus</i>	500	21	No improvement	
Moderate Severity						
R. R.	Acute tonsillitis	Hemolytic <i>Strepto-</i> <i>coccus</i>	250	7	Improved	
R. E.	Cellulitis	<i>Staph. aureus</i>	250	4	Dramatic recovery	
R. B.	Cellulitis, nose	<i>Staph. aureus</i>	500	10	Dramatic recovery	
E. I.	Foot infection with bronchopneumonia	<i>Staph. aureus</i>	500	18	Recovery	
M. M.	Bilateral cellulitis		500	5	Recovery	
H. F.	Cellulitis, forehead	<i>Staph. aureus</i>	500	7	Recovery	
E. F.	Cellulitis, ankle	Hemolytic <i>Strepto-</i> <i>coccus</i>	250	3	Dramatic recovery	Mild pruritus ani
L. H.	Acute throm- bophlebitis		250	5	Recovery	
Severe Infections						
C. D.	Cellulitis, face	<i>Staph. aureus</i>	500	7	Dramatic recovery	
J. S.	Empyema, post-shot	<i>Staph. aureus</i>	500	14	Recovery	
D. S.	Breast abscess	<i>Staph. aureus</i>	500	7	Drained surgically	
					Dramatic recovery	
A. H.	Breast cellulitis		500	14	Recovery by resolution	

ester and erythromycin base; however, in rats, Lee<sup>18</sup> has shown a slower and more limited excretion of the propionyl erythromycin in the bile than with erythromycin base. This may explain the higher blood levels obtained with the propionyl ester.

The early onset of serum concentrations with the capsules of propionyl erythromycin is of value in treating acute infections, because of the greater rapidity of onset of therapeutic activity. The higher levels, besides producing increased penetration into the sites of infection, permit therapeutic concentrations in subjects who otherwise might not obtain antibacterial levels with other preparations of erythromycin. The longer duration of antibacterial effect increases the safety of the medication by maintaining antibiotic effect over a greater period of time in those patients who forget a dose when it is due.

Most of the propionyl erythromycin absorbed is believed to remain in the form of the propionyl ester, since physiochemical studies performed on the urine reveal that 80 per cent is excreted as the propionyl ester.

Jones and Finland<sup>19</sup> and Garrod<sup>20</sup> have pointed out the superior in vitro activity of erythromycin against pathogenic bacteria when this antibiotic is compared to oleandomycin and spiramycin. Finland has recently reported that triacetyloleando-

mycin did not produce so satisfactory antibiotic activity as did propionyl erythromycin.<sup>21</sup>

Therapeutic action of an antibiotic is difficult to evaluate unless extensive comparative studies are performed. The patients treated with propionyl erythromycin had infections typical of those seen in outpatient or hospital. They responded satisfactorily to propionyl erythromycin. The only failure occurred in a patient with hydradenitis suppurativa. This patient had also failed to respond to other antibiotics, apparently because of the marked fibrosis present. The condition had existed for several years. The propionyl erythromycin was considered highly effective in the rest of the cases studied.

No serious side effects were encountered during therapy in the 23 cases of infection or 12 patients receiving prolonged therapy. Two instances of mild pruritus ani occurred, and 1 patient noted a "loamy" taste. One patient who complained of upset stomach continued to have the same symptoms when an identical placebo was substituted at the end of her therapy.

#### CONCLUSIONS

1. Propionyl erythromycin is equally active in vitro as erythromycin base. Their spectra are practically the same. The potency against bacteria in the spectra are identical.

2. Propionyl erythromycin produces earlier, higher, and more prolonged high levels than does erythromycin base in capsules, erythromycin base in tablets protected by an acid-resistant coating, triacetyloleandomycin, and triacetyloleandomycin with glucosamine.

3. Clinical studies demonstrated that propionyl erythromycin is an effective antibiotic.

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#### BIBLIOGRAPHY

1. MCGUIRE, J. M.; BUNCH, R. L.; ANDERSON, R. C.; BOAZ, H. E.; FLYNN, E. H.; POWELL, H. M., AND SMITH, J. W.: "Ilotycin," a new antibiotic, *Antib. & Chemo.* 2:281, June, 1952.
2. HERRELL, W. E.: Erythromycin in infectious diseases, *M. Clin. North America*, March, 1954, p. 569.
3. HERRELL, W. E.: Erythromycin, New York, Medical Encyclopedia, Inc., 1955.
4. SHOEMAKER, E. H., AND YOW, E. M.: A clinical evaluation of erythromycin, *A.M.A. Arch. Int. Med.* 93:397, March, 1954.
5. SMITH, J. W.; DYKE, R. W., AND GRIFFITH, R. S.: Erythromycin: Observations on serum levels after oral administration and on the treatment of thirty-three patients, *Proc. Central Soc. Clin. Research* 25:80, 1952.
6. HAIGHT, T. H., AND FINLAND, M.: Laboratory and clinical studies on erythromycin, *New England J. Med.* 247:227, Aug. 14, 1952.
7. GRIGSBY, M. E.; JOHNSON, J. B., AND SIMMONS, G. W.: Some laboratory and clinical experiences with erythromycin, *Antib. & Chemo.* 3:1029, Oct., 1953.
8. WADDINGTON, W. S.; MAPLE, F. M., AND KIRBY, W. M.: Treatment of bacterial pneumonia with erythromycin, *A.M.A. Arch. Int. Med.* 93:556, April, 1954.
9. ROMANSKY, M. J.; NASOU, J. P.; DAVIS, D. S., AND RITTS, R. E., JR.: Clinical spectrum of erythromycin as evaluated in two hundred sixty patients, *J.A.M.A.* 164:1197, July 13, 1957.
10. FORFAR, J. O., AND MACCABE, A. F.: Erythromycin—a review. *In: Antibiotics et Chemotherapy*, Vol. 4. Basil, S. Karger. 1957, p. 115.

11. HEILMAN, F. R.; HERRELL, W. E.; WELLMAN, W. E., AND GERACI, J. E.: Some laboratory and clinical observations on a new antibiotic, erythromycin (Ilotycin), *Proc. Staff Meet., Mayo Clin.* 27:285, July 16, 1952.
12. MURPHY, H. W.: Esters of erythromycin. I. Some water-insoluble, substantially tasteless esters. *In: Antibiotics Annual 1953-1954*, New York, Medical Encyclopedia, Inc., 1954, p. 500.
13. STEPHENS, V. C.: Esters of erythromycin. II. Derivatives of cyclic anhydrides. *In: Antibiotics Annual 1953-1954*, New York, Medical Encyclopedia, Inc., 1954, p. 514.
14. STEPHENS, V. C., AND CONINE, J. W.: Esters of erythromycin. III. Esters of low molecular weight aliphatic acids. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 346.
15. WILEY, P. F.; GERZON, K.; FLYNN, E. H.; SIGAL, M. V., JR.; WEAVER, O.; QUARCK, U. C.; CHAUVETTE, R. R., AND MONAHAN, R.: Erythromycin. X. Structure of erythromycin, *J. Am. Chem. Soc.* 79:6062, 1957.
16. GRIFFITH, R. S.; STEPHENS, V. C.; WOLFE, W. A.; BONIECE, W. S., AND LEE, C.-C.: Preliminary studies on propionyl erythromycin, *AM & CT* 5:609, Oct., 1958.
17. WOLFE, R. N.: Personal communication.
18. LEE, C.-C.: Personal communication.
19. JONES, W. F., AND FINLAND, M.: Antibiotic combinations. Tetracycline, erythromycin, Oleandomycin and spiramycin, and combinations of tetracycline with each of the other three agents. Comparisons of activity in vitro and antibacterial action of blood after oral administration, *New England J. Med.* 257:481, Sept. 12, 1957.
20. GARROD, L. P.: The erythromycin group of antibiotics, *Brit. M. J.* 2:5036, July 13, 1957.
21. KUNIN, C. N.; PRYLES, C. V., AND FINLAND, M.: Antibacterial activity of serum after oral doses of triacetyloleandomycin, erythromycin, potassium penicillin V and penicillin V, *Pediatrics* 22:422, Sept., 1958.

# Clinical and Laboratory Studies of Erythromycin Propionate

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Erythromycin, in six years of clinical use, has become established as an effective antibiotic. However, there have been difficulties in obtaining an oral dosage form that gives consistent blood levels, because erythromycin is inactivated by gastric secretions.<sup>1-3</sup>

Erythromycin base in gelatin capsules and in uncoated tablets produced generally low, irregular, and, in some instances, undetectable blood levels.<sup>1, 3-6</sup> The uncoated tablets gave better levels when given to patients with pernicious anemia<sup>1</sup> and when given with aluminum hydroxide gel.<sup>3</sup> Heavily coated enteric tablets also gave better levels but were absorbed irregularly and slowly.<sup>2, 5</sup> Josselyn found that film sealed tablets and an aqueous suspension of erythromycin stearate gave early and high levels.<sup>5, 7</sup>

"Specially coated" tablets were found to produce levels comparable to uncoated tablets given with aluminum hydroxide gel<sup>3</sup> or when given to patients with pernicious anemia.<sup>1</sup> Subsequently, others have found that although the specially coated tablets produced adequate levels in most instances, there was still considerable variability, and a few individuals showed low or nondetectable responses.<sup>3, 8-11</sup>

Recently, a chemical modification, a propionyl ester of the erythromycin base, was made available. This paper deals with laboratory and clinical studies of this ester, hereafter referred to as erythromycin propionate.\* Triple crossover studies were carried out in healthy volunteers, comparing erythromycin base in a gelatin capsule, erythromycin base in a commercially available coated tablet,<sup>†</sup> and a gelatin capsule of erythromycin propionate. In addition, 20 patients with acute respiratory infections were treated with erythromycin propionate, and the clinical response and tolerance to the medication were noted.

## METHODS AND MATERIALS

*Measurement of Serum Levels.* A comparison of the serum antistreptococcal activity was made in 13 subjects after ingestion of 250 mg. of erythromycin base in a coated tablet and 250 mg. of erythromycin propionate in a gelatin capsule. In 12 of the same subjects, the serum activity was compared after ingestion of a 250 mg. erythromycin propionate capsule and 250 mg. of erythromycin base in a gelatin capsule.

The subjects were normal young adults who ranged in age from 22 to 35 years old and in weight from 122 to 175 pounds. Each subject received a 250 mg. oral dose of the test antibiotic after an overnight fast of nine hours, with an interval of two or more days between doses. Blood was drawn one-half, one, two, four, six, and eight hours after the medication was swallowed. The subjects were permitted to eat after the two hour blood was drawn. At least one hour was allowed for clot re-

\* The trade name of Eli Lilly & Co. for erythromycin propionate is Ilosone.

† The trade name of Eli Lilly & Co. for erythromycin coated tablet is Ilotycin #23.

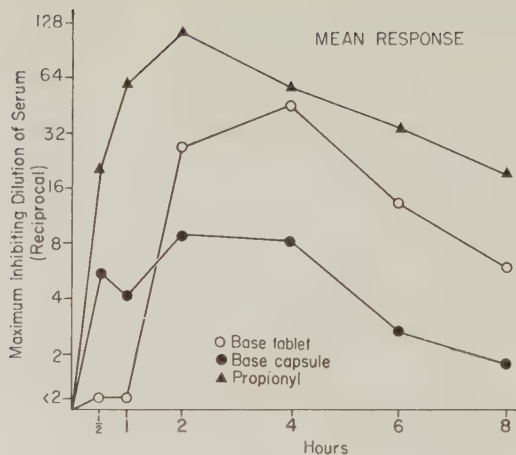


FIG. 1. Average serum inhibiting levels for the test organism in 13 volunteers after ingestion of 250 mg. of erythromycin base in a specially coated tablet, erythromycin base in a gelatin capsule, and erythromycin propionate in a gelatin capsule.

traction before the specimens were centrifuged; the serum was then stored at  $-20^{\circ}\text{C}$ . until the time of the assay. Each subject's sera, from both days of the comparison study, were assayed simultaneously to minimize laboratory variability.

Serum levels were determined by a modification of the penicillin assay technique of Rammelkamp,<sup>12</sup> as described by Ziegler and McGuire,<sup>13</sup> using a group A *Streptococcus* as the test organism. Twofold dilutions of the serum were made in Tryptose phosphate broth ( $\text{pH } 7.0$ ). The inoculum consisted of 0.04 ml. of a  $10^{-3}$  dilution of an 18 to 20 hour culture of the organism. The tubes were examined for growth after incubation for 18 hours at  $37^{\circ}\text{C}$ . The end point was the highest dilution of the unknown serum in which there was no macroscopic evidence of growth, i.e., there was no hemolysis and the erythrocytes that had settled to the bottom of the tube remained bright red. When this tube was agitated and streaked, none to a few colonies of streptococci were cultured from each loopful of broth. The inoculum used in each test, as determined by plate count each day, was 50 to 100 thousand organisms/ml. The antibiotic activity of each specimen of serum was expressed as the maximum dilution of serum that yielded no growth. Sensitivity of the test organism to a standard solution of erythromycin was also determined each day using

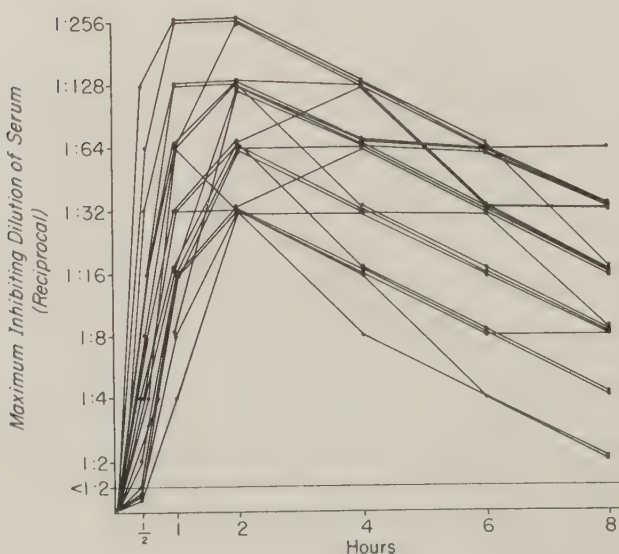
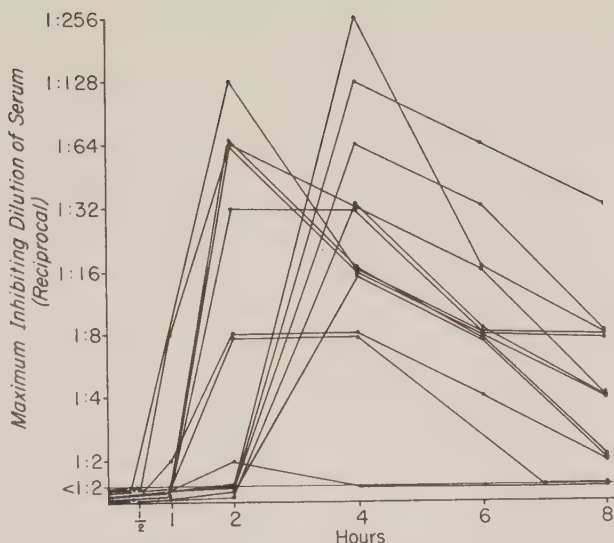


FIG. 2. Inhibiting serum levels in 22 subjects after ingestion of 250 mg. of erythromycin propionate. There was prompt absorption, with high levels, in every instance.

FIG. 3. Inhibiting serum levels in 13 subjects after ingestion of 250 mg. of erythromycin in a specially coated tablet. Absorption was delayed and the results were variable.



the same broth and inoculum. In these control tests, .02  $\mu\text{g.}/\text{ml.}$  of erythromycin base and also of erythromycin propionate consistently inhibited growth.

Serum levels were also determined in 5 patients with acute respiratory infections after at least eight 0.5 Gm. doses of erythromycin propionate at six hour intervals. Four patients had pneumococcal pneumonia and 1 a lung abscess. For comparison, in 2 of these same patients serum levels were determined after at least eight 0.5 Gm. doses of erythromycin in a specially coated tablet. Blood samples were drawn 1, 2, 4, and 6 hours after the 9 A.M. dose on the day of the test.

*Clinical Trial.* Twenty patients admitted to the King County Hospital infectious disease ward were treated with erythromycin propionate. Eighteen had acute bacterial pneumonia, 1 had acute bronchitis, and 1 a lung abscess. There were 14 men and 6 women. Ten were chronic alcoholics, and their ages ranged from 17 to 87 years old. All patients received 0.5 Gm. of the drug every six hours, and the duration of therapy in most instances was five to seven days. The patients were seen daily to evaluate the clinical response and to note side effects.

## RESULTS

*Laboratory Results.* The average serum levels of antistreptococcal activity after the oral administration of the three erythromycin preparations are presented in

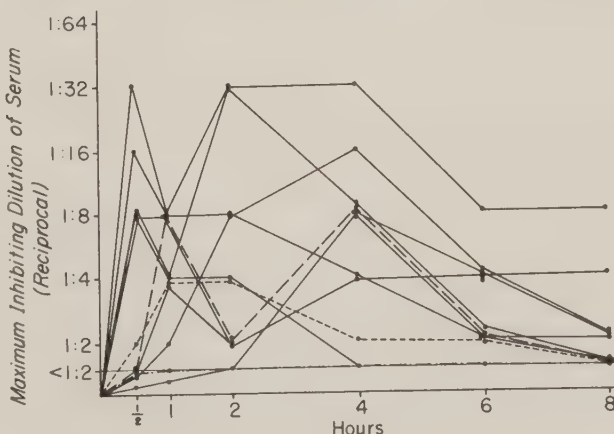


FIG. 4. Inhibiting serum levels in 11 subjects after ingestion of 250 mg. of erythromycin in a gelatin capsule.

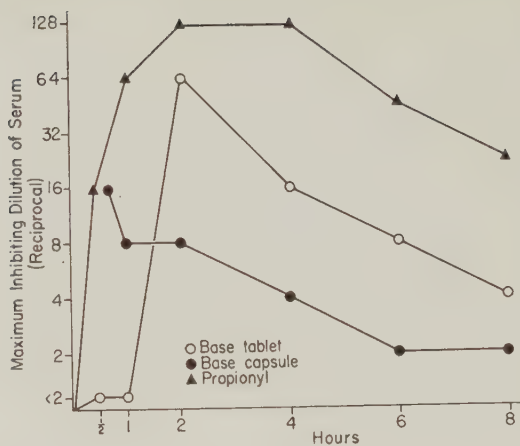


FIG. 5. Serum levels in one subject (case 4) with all three dosage forms. Eight subjects showed this type of response.

figure 1. Erythromycin propionate produced high serum levels within the first hour after administration, and the levels were higher than those with the other preparations at all time intervals measured. For example, at the end of the second hour, the erythromycin propionate level was 200 per cent higher than that obtained with the specially coated tablets. Also, from the slope of the curve, it appears that its activity was sustained longer than the other preparations.

To illustrate further the differences, all the results obtained with each preparation are shown separately. Figure 2 shows the results of 22 assays of serum activity after oral administration of erythromycin propionate. These were done in 13 subjects, 9 of whom received the antibiotic on two separate occasions. There was a prompt, consistently high level of activity in all subjects. The regularity of the response was demonstrated in that 20 of the assays showed peak values at one or two hours, and all but four had significant activity at one-half hour. Figure 3 shows the results of 13 assays of serum activity after administration of the commercially available specially coated tablets. The variability of the response, the lower levels of activity obtained, and the longer interval required for peak values to be reached are apparent. This response is in agreement with the results of earlier studies.<sup>1, 3, 8-10</sup> Figure 4 shows the results of 11 assays of serum activity after administration of erythromycin base in capsules. Here the lowest levels of activity were produced, as expected, and again a wide variability of response was noted.

An analysis of results in individual subjects showed three general patterns. Subject 4 (fig. 5) is typical of 8 of the 13 crossover studies. This shows the prompt, predictable, high level of activity produced by erythromycin propionate and the de-

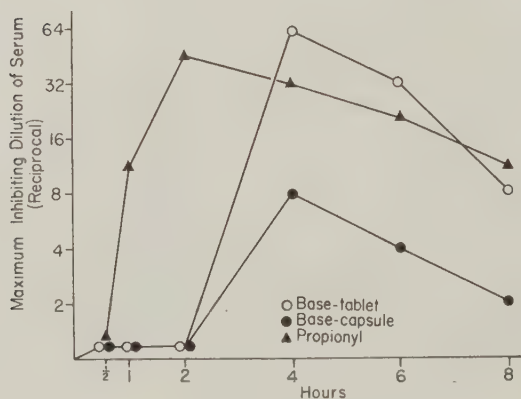
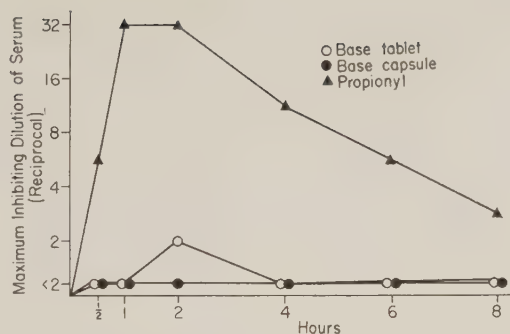


FIG. 6. Four subjects showed this type of response. (Case 1.)

FIG. 7. This "poor absorber" (case 8) had good serum levels only with erythromycin propionate.



layed lower level obtained with the specially coated tablet and the base capsule. Subject 1 (fig. 6) is typical of 4 of the 13 studies. The peak value for the specially coated tablet was slightly higher than that for erythromycin propionate, but a much longer interval was required to obtain this. When the total antibacterial activity of the two preparations was compared, by measuring the areas subtended under the curves, the erythromycin propionate produced significantly greater activity. Subject 8 (fig. 7) shows the results in the one individual who was a "poor absorber." This subject had no detectable level at any time with the erythromycin base in a capsule and only one low level of activity with the specially coated tablet. However, the erythromycin propionate produced prompt, effective serum levels.

High serum levels were maintained at all intervals assayed in the 5 patients receiving erythromycin propionate. In the 2 patients in whom crossover studies were carried out, the serum levels were more than 100 per cent higher than with the tablet.

*Clinical Results.* The response of 20 patients treated with erythromycin propionate is presented in table I. Patients with uncomplicated pneumonia generally responded promptly to therapy. Only 13 patients had definite bacteriological evidence of pneumococci, but it was probable that 4 others also had pneumococcal pneumonia. In 12 of the 17 patients there was definite symptomatic improvement in the first two days, and they were all afebrile in from two to six days.

In 2 patients whose responses were regarded as indeterminate, there were early signs of clinical improvement, but their temperatures remained elevated for 10 to 12 days. In each there was a probable reason for the lack of immediate response. One patient had partial collapse of the right middle lobe and another had a sterile pleural effusion. A third patient developed infected lung cysts and remained febrile for more than three weeks. All 3 were treated with penicillin at a later date. The remaining patient with an indeterminate response had a slowly resolving left lower lobe pneumonia and is still under study.

There was one apparent treatment failure—a 44 year old white woman who had persisting fever and purulent sputum for three weeks. In the third week she was treated with penicillin and promptly became afebrile. She had had a similar illness

TABLE I  
*Clinical Results in 20 Patients Treated with Erythromycin Propionate*

	Total no.	Good	Indeterminate	Poor
Pneumococcal pneumonia	17	12	4	1
<i>Klebsiella</i> pneumonia	1	0	1	0
Acute bronchitis	1	1	0	0
Lung abscess	1	1	0	0

one year previously. The patient with the lung abscess was treated for 28 days, although he became afebrile with decrease in cough and sputum in five days.

In the 20 patients evaluated, the response to therapy was similar to our earlier results with erythromycin and the tetracyclines and similar to the experience of others.<sup>8, 9, 14-18</sup>

*Side Effects.* All patients tolerated the medication well. No nausea and vomiting or other side effects were noted in this series. The patient with the lung abscess was treated with 2 Gm./day for 28 days without any apparent adverse effects.

#### COMMENTS

The results of these studies indicate that erythromycin propionate was better absorbed than the other dosage forms tested. In vitro, the minimal inhibitory concentrations of erythromycin propionate and erythromycin base were equal, indicating that the ester had the same degree of activity as the base. Therefore, the greater serum activity obtained in all subjects was due to more complete absorption of erythromycin in an active form. In addition to better absorption, erythromycin propionate was more uniformly absorbed. In the past, widely variable blood levels have been noted with coated tablets. This did not occur with erythromycin propionate. The responses were consistent and predictable, and one individual, who was a "poor absorber" with the base, showed good levels with erythromycin propionate. Earlier levels were obtained with erythromycin propionate than with the specially coated tablets in all of the subjects, and most subjects showed high levels at one-half hour. This suggests that the ester was absorbed readily from the stomach as well as from the upper small intestine.

The results of the clinical trials showed that erythromycin propionate had effective antibacterial action in vivo. The responses were comparable to our own past results with erythromycin base and to the results reported by others.<sup>8, 9, 14-18</sup>

We did not observe gastrointestinal irritation in any of the patients, and 1 tolerated the drug well for four weeks. Erythromycin propionate thus appears to be at least as well tolerated as the dosage forms now being used.

#### SUMMARY

In 13 triple crossover studies, erythromycin propionate in capsules produced earlier, higher, and more prolonged serum levels than erythromycin base in capsules or in specially coated tablets. In addition, the propionyl ester was more uniformly absorbed and produced a more predictable response than the other forms.

In 18 patients with pneumonia, 1 with a lung abscess and 1 with bronchitis, the medication was well tolerated, produced consistent serum levels, and the clinical responses were comparable to earlier results obtained with erythromycin and other antibiotics.

#### BIBLIOGRAPHY

1. SMITH, J. W.; DYKE, R. W., AND GRIFFITH, R. S.: Absorption following oral administration of erythromycin, *J.A.M.A.* 151:805, 1953.
2. GRIFFITH, R. S.: Blood levels and clinical results with erythromycin. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 269.
3. JOSSELYN, L. E., AND SYLVESTER, J. C.: Absorption of erythromycin, *Antib. & Chemo.* 3:63, 1953.
4. HEILMAN, E. R.; ET AL: Laboratory and clinical observations on a new antibiotic, erythromycin (Ilotycin), *Proc. Staff Meet., Mayo Clin.* 27:285, 1952.

5. JOSSELYN, L. E.; EDINCOTT, C., AND SYLVESTER, J. C.: Absorption of erythromycin. III. Various pharmaceutical forms. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 279.
6. HAIGHT, T. H., AND FINLAND, M.: The antibacterial action of erythromycin, *Proc. Soc. Exper. Biol. & Med.* 81:175, 1952.
7. SYLVESTER, J. C., AND JOSSELYN, L. E.: Absorption of erythromycin. II. Erythromycin stearate, *Antib. & Chemo.* 3:930, 1953.
8. KIRBY, W. M. M.; MAPLE, F., AND O'LEARY, B.: Erythromycin serum concentrations following administration in acid resistant tablets, *Antib. & Chemo.* 3:473, 1953.
9. GRIGSBY, M. E.; JOHNSON, J. B., AND SIMMERS, G. W.: Some laboratory and clinical experiences with erythromycin, *Antib. & Chemo.* 3:1029, 1953.
10. WELCH, H.: *Principles and Practices of Antibiotic Therapy*, New York, Medical Encyclopedia, Inc., 1954, p. 177.
11. JONES, W. F., AND FINLAND, M.: Antibiotic combinations: Antistreptococcal and anti-staphylococcal activity of plasma of normal subjects after ingestion of erythromycin or penicillin or both, *New England J. Med.* 255:1019, Nov. 29, 1956.
12. RAMMELKAMP, C. H.: A method for determining the concentrations of penicillin in body fluids and exudates, *Proc. Soc. Exper. Biol. & Med.* 51:95, Oct., 1942.
13. ZIEGLER, D. W., AND MCGUIRE, J. M.: A bioassay for the determination of "Ilotycin" in serum and other body fluids, *Antib. & Chemo.* 3:67, 1953.
14. HAIGHT, T. H., AND FINLAND, M.: Laboratory and clinical studies of erythromycin, *New England J. Med.* 247:227, 1952.
15. AUSTRIAN, R., AND ROSENBLUM, R.: The relative efficacy of erythromycin (Ilotycin) and of penicillin in the treatment of pneumococcal lobar pneumonia, *Am. J. Med. Sc.* 226:487, 1953.
16. GIBSON, C. D., JR.; NASHAN, H., AND ANDERSON, D. M.: The treatment of bacterial pneumonia: a controlled clinical study, *Ann. Int. Med.* 41:112, 1954.
17. MOULTON, B.; BLATT, N. H., AND LEPPER, M. H.: Use of erythromycin in the treatment of common respiratory infections, soft tissue infections and complications of exanthematous fevers. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., p. 229.
18. ROMANSKY, M. J.; NASOU, J. P.; DAVIS, D. S., AND RITTS, R. E., JR.: Clinical spectrum of erythromycin as evaluated in two hundred sixty patients, *J.A.M.A.* 164:1197, 1957.

# Absorption of Erythromycin Propionate and Triacetyloleandomycin

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A comparison of the antistreptococcal and antistaphylococcal activity of serum obtained after the oral administration of coated tablets of erythromycin and capsules of triacetyloleandomycin in adults and of erythromycin ethyl carbonate and triacetyloleandomycin in children was recently reported from this laboratory.<sup>1</sup> The antibacterial activity of the two agents given in this manner was quite similar. The peak of activity, however, occurred somewhat later in the adults after erythromycin than after triacetyloleandomycin.

A supply of erythromycin propionate became available after that study was completed. The same adult subjects were therefore given an equivalent dose of that agent and studied in the same manner. Absorption of this preparation was much more rapid than for coated erythromycin and serum levels of antibacterial activity were found to be higher than for either the coated erythromycin tablets or the capsules of triacetyloleandomycin.

Subsequently, a direct comparison was made of the absorption and serum antibacterial activity of erythromycin propionate and triacetyloleandomycin. Eight other normal men were given an oral dose of each drug, equivalent to 500 mg. of base activity. Serum antibacterial activity was assayed by the serial twofold dilution method using *Streptococcus* 98 and *Staphylococcus* 209P as in the earlier study. Results were expressed as the reciprocal of the maximum inhibiting dilution of serum. The sera were also tested by the cup-plate method using *Sarcina lutea* in the Research Laboratories of the Eli Lilly & Co. and the results expressed in terms of erythromycin activity in  $\mu\text{g./ml.}$

Curves representing the mean serum antibacterial activity at stated times up to 25 hours showed that the peak absorption of each drug was achieved at about two hours after the oral dose was given. By the end of 25 hours very little activity of either preparation was present, although some antistreptococcal activity of erythromycin was still detectable. For each organism and at each period, the mean activity of erythromycin propionate was found to be greater than for triacetyloleandomycin by a factor ranging from  $2\frac{1}{2}$  to 8 fold. The activity expressed as  $\mu\text{g./ml.}$  of erythromycin showed a similar relationship.

Expressed numerically as the mean values, both in terms of peak levels and total activity, erythromycin propionate was found to yield significantly higher activity. There was somewhat greater variation in erythromycin activity as compared to that of oleandomycin, but the magnitude of the difference between the drugs was so great that this was of negligible importance and the differences observed were statistically highly significant.

Thus a crossover study in normal men demonstrated that erythromycin propionate orally produced  $2\frac{1}{2}$  to 8 fold higher levels of activity against a *Streptococcus*, a *Staphylococcus*, and *S. lutea* than an equivalent dose of triacetyloleandomycin. Compared with previous data on the absorption of coated tablets of erythromycin, the propionate was found to be more rapidly absorbed and to produce consistently higher levels.

## BIBLIOGRAPHY

1. KUNIN, C. M.; PRYLES, C. V., AND FINLAND, M.: Antibacterial activity of serum after oral doses of triacetyloleandomycin, erythromycin, potassium penicillin V and penicillin V, *Pediatrics* 22:422-431, Sept., 1958.

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# Laboratory and Clinical Studies of Intramuscular Erythromycin

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Erythromycin is an antibiotic of established effectiveness when given orally. The inability of certain patients to receive oral medication prompted the development of a parenteral preparation of this drug. Laboratory studies by the manufacturer revealed that, of the various derivatives of erythromycin investigated, the ethyl succinate salt, dissolved in propylene glycol, was the most desirable from the standpoint of nonirritating qualities and from other pharmacological data.

The present investigation of this form of intramuscular erythromycin\* was divided into two phases, one to determine concentrations in the peripheral blood after its injection and one to determine its clinical effectiveness in various types of acute, gram-positive infections.

*Blood Level Studies.* Three separate groups of subjects were included in this part of the study, two groups involving adults and one involving children.

Group I included 9 healthy adults each of whom received intragluteal injections of erythromycin ethyl succinate equivalent to 100 mg. of erythromycin activity at six hour intervals over a 24 hour period. Blood samples for assay were obtained at 0, 1, 2, 4, 6, and 24 hours after the first injection. The results are shown in table I.

Group II included 20 healthy adults each of whom received a single intragluteal injection of erythromycin ethyl succinate equivalent to 100 mg. of erythromycin activity. Blood samples were taken at 0, 4, 8, 12, and 16 hours after injection and assayed for erythromycin content. The results are shown in table II.

Group III was comprised of 20 children, weighing between 50 and 75 lb., each of whom received a single intragluteal injection of erythromycin ethyl succinate equivalent to 50 mg. of erythromycin activity. From 4 children a single blood sample was drawn at 1 hour after injection, from 4 others a single blood sample was taken at 2 hours after injection, and so on at 4, 6, and 12 hours using individual groups of 4 children each for each time interval. These results are shown in table III.

These various blood level findings revealed that erythromycin ethyl succinate is rapidly absorbed in both adults and children after intragluteal injection. Moreover, definite concentrations of the drug remain in the blood for at least eight hours in adults and often for at least 12 hours in both adults and children.

*Clinical Studies.* A total of 55 hospital patients with predominantly gram-positive types of infections were treated with intramuscular erythromycin ethyl succinate. These patients were located on medical and surgical wards and received supportive care but no other antimicrobial therapy during the study. One group of 15 patients was treated with 100 mg. of the drug intramuscularly every six hours until the infection either responded or failed to respond. The remaining 40 patients received an injection of 100 mg. every 12 hours for a maximum of three days. This latter schedule was instituted after blood level findings had indicated that erythromycin

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\* The trade name of Abbott Laboratories for intramuscular erythromycin is Erythrocin I.M. Intramuscular erythromycin used in this study was provided by this firm.

TABLE I  
*Erythromycin Blood Levels in Adults after the Intramuscular  
Injection of 100 mg. Each Six Hours*

Subject	$\mu$ g. erythromycin per ml. serum					
	Hours after first injection					
	0	1	2	4	6	24
J. A. G.	<0.02	0.64	0.64	0.08	0.08	0.64
J. G.	<0.02	0.64	<0.02	0.32	0.32	0.64
A. M.	<0.02	0.64	—*	0.64	0.64	2.56
H. M.	<0.02	0.64	1.28	2.56	2.56	1.28
M. D.	<0.02	0.64	0.64	0.32	0.32	1.28
C. C.	<0.02	1.28	1.28	0.64	0.64	—
J. S.	<0.02	1.28	2.56	2.56	2.56	5.12
H. W.	<0.02	1.28	0.64	0.64	1.28	1.28
W. B.	<0.02	0.64	0.64	0.64	0.64	—
Median value	<0.02	0.64	0.64	0.64	0.64	1.28

\* No specimen obtained.

activity persisted in many persons for at least 12 hours after a single injection of 100 mg.

Each patient was medicated and closely supervised by one of the resident physicians on the hospital ward. The response of the infectious process was watched closely and evidences of pain or other untoward sequelae resulting from the injection

TABLE II  
*Erythromycin Blood Levels in Adults after a Single  
Intramuscular Injection of 100 mg.*

Subject	$\mu$ g. erythromycin per ml. serum				
	Hours after injection				
	0	4	8	12	16
D. G.	<0.02	0.32	0.04	<0.02	<0.02
M. L.	<0.02	0.64	0.04	<0.02	<0.02
T. C.	<0.02	0.64	0.08	0.02	<0.02
M. D.	<0.02	0.64	0.16	0.02	<0.02
R. P.	<0.02	0.64	0.08	<0.02	<0.02
M. V.	<0.02	0.16	0.04	<0.02	<0.02
E. T.	<0.02	0.64	0.04	<0.02	<0.02
R. C.	<0.02	0.32	0.08	0.02	<0.02
S. G.	<0.02	0.32	0.08	<0.02	<0.02
E. T.	<0.02	0.32	0.16	0.04	<0.02
G. B.	<0.02	0.32	0.08	<0.02	<0.02
J. W.	<0.02	0.64	0.32	0.08	0.04
L. B.	<0.02	0.64	0.16	0.02	<0.02
R. B.	<0.02	0.32	0.08	0.02	<0.02
C. L.	<0.02	0.32	0.08	0.02	<0.02
R. A.	<0.02	0.64	0.16	0.02	<0.02
A. H.	<0.02	0.32	0.16	0.04	<0.02
E. K.	<0.02	0.64	0.08	0.02	<0.02
B.	<0.02	0.64	0.16	0.02	<0.02
M.	<0.02	0.32	0.04	<0.02	<0.02
Median value	<0.02	0.64	0.08	0.02	<0.02

TABLE III

*Erythromycin Blood Levels in Children after an Intramuscular Injection of 50 mg. (at Each Time Interval a Different Group of 4 Subjects Was Used)*

	$\mu$ g. erythromycin per ml. serum				
	Hours after injection				
	1	2	4	6	12
	0.64	1.28	0.32	0.08	0.02
	0.64	0.64	0.64	0.04	0.04
	0.64	2.56	0.64	0.08	<0.02
	1.28	0.64	0.32	0.08	<0.02
Median value	0.64	0.64	0.32	0.08	<0.02

TABLE IV

*Summary of Data from Patients Treated with Intramuscular Erythromycin*

Number of patients	Clinical diagnosis	Clinical response		Pain on injection		
		Good	Poor	Marked	Mild	None

<i>Treatment—100 mg. Every Six Hours for an Indefinite Period*</i>						
12	Pneumonia	9	3			
1	Abscess	0	1			
1	Cellulitis	1	0			
1	Pelvic inflammatory disease	1	0			
				4	3	8
15		11	4	4	3	8

<i>Treatment—100 mg. Every 12 Hours for a Maximum of Three Days†</i>						
17	Cellulitis	15	2			
2	Pneumonia	2	0			
13	Abscess	9	4			
2	Bronchitis	2	0			
1	Tonsillitis	1	0			
2	Burn	2	0			
1	Paronychia	1	0			
1	Tenosynovitis	1	0			
1	Decubitus ulcer	1	0			
				3	17	20
40		34	6	3	17	20

\* The organisms isolated from these cases and the total number of times they were isolated were: beta-hemolytic streptococci, 5; *Staphylococcus aureus* (coagulase-positive), 2 (these two organisms were sensitive in vitro to erythromycin and tetracycline and resistant to penicillin); *Diplococcus pneumoniae*, 3; no pathogens isolated, 4; no specimen obtained, 1.

† The organisms isolated from these cases and the total number of times they were isolated were: beta-hemolytic streptococci, 8; *Streptococcus viridans*, 1; nonhemolytic streptococci, 1; *Staphylococcus aureus* (coagulase-positive), 21; *D. pneumoniae*, 1; *Escherichia coli*, 3; *Proteus*, 2; *Pseudomonas*, 1; no pathogens isolated, 2; no specimen obtained, 8. The in vitro sensitivities of these 21 staphylococci were as follows: erythromycin, 18 sensitive, 3 resistant; tetracycline, 14 sensitive, 7 resistant; chloramphenicol, 20 sensitive, 1 resistant; penicillin, 10 sensitive, 11 resistant.

were looked for and recorded. Cultures for bacteriological study were taken before treatment except when specimens were not obtainable. Antibiotic sensitivity tests were performed with staphylococcal isolates using a standard disc technique. These tests were not performed on beta-hemolytic group A streptococci or on pneumococcus isolates for obvious reasons, or on gram-negative isolates.

The results of this treatment are shown in table IV. It is readily apparent that the two injection/day schedule was just as effective as the more rigorous regimen. Of the total of 55 patients treated, 45 had a good response, i.e., defervescence of acute illness (temperature, erythema, swelling, pain), within 48 to 96 hours. The 10 patients with poor responses were mainly those with infections due to erythromycin-insensitive organisms or gram-negative organisms. About one half of the patients experienced pain at the site of injection, but in only 7 was the pain marked.

#### DISCUSSION

The availability of an intramuscular form of erythromycin greatly enhances the usefulness of this effective antibiotic. The present study demonstrates that the ethyl succinate salt of this drug can be injected intramuscularly with subsequent rapid absorption in both adults and children. Demonstrable amounts of the drug remain in the circulation of many persons for at least 12 hours after a single injection of this preparation. Moreover, the injection itself causes relatively little local discomfort, and no general toxicity has been noted.

Various gram-positive acute infections in adults responded rapidly to a dosage schedule of 100 mg. of drug injected twice daily. Compared to previous experience with other intramuscular antibiotics in this type of disease, it is our consensus that injectable erythromycin is equally effective against sensitive organisms. An important use of this preparation undoubtedly will be for the treatment of staphylococcal infections, many of which are resistant to one or more other antimicrobial agents. In the patient unable to receive oral medication, erythromycin ethyl succinate should prove to be a valuable adjunct in the management of gram-positive infections.

#### CONCLUSIONS

1. Erythromycin ethyl succinate for intramuscular injection has been investigated in regard to absorption, persistence of circulating drug in the blood, and clinical effectiveness in patients with gram-positive types of infections.

2. Intragluteal injection of 100 mg. of the drug in adults or 50 mg. in children resulted in rapid absorption and maintenance in many persons of measurable concentrations in the blood for at least 12 hours.

3. Fifty-five adult patients with acute infections were treated with intramuscular erythromycin, and in 45 the response was rapid. The infections that did not respond were due mainly to erythromycin-resistant organisms or gram-negative organisms.

4. A dosage of 100 mg. intramuscularly every 12 hours was effective, and local discomfort due to the injection was minimal.

5. In gram-positive types of infections, and perhaps especially in staphylococcal infections, this form of erythromycin should prove to be of considerable value as an added method of treatment in the acutely ill patient.

# Novobiocin in the Treatment of Surgical Infections

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This report deals with the clinical effectiveness of novobiocin\* in 130 consecutive patients with a variety of surgical infections, due largely to coagulase-positive hemolytic staphylococci. Fifty-one patients were treated with this antibiotic exclusively, and 79 had received prior ineffective therapy with other antibiotics. The conditions treated will be discussed under the following classifications: (1) infections complicating peripheral vascular disease; (2) soft-tissue infections, primary and secondary; (3) pulmonary infections; and (4) miscellaneous infections.

## ROUTE OF ADMINISTRATION AND DOSAGE

*Oral Administration.* As a loading dose, 500 mg. was given orally followed by 1 Gm./day in four equally divided doses. With this dose, plasma concentrations of 6 to 46  $\mu\text{g./ml.}$  were maintained.<sup>1</sup> The duration of therapy was based on both the clinical and bacteriological response. In most cases, the drug was given for five to seven days.

*Intravenous Administration.* Twenty-one gravely ill patients were unable to tolerate the oral medication or required prompt massive therapy. In most cases, the parenteral route was discontinued when the drug could be given orally. For intravenous use, 500 mg. of crystalline monosodium novobiocin, dissolved in 50 to 500 ml. of 5 per cent dextrose or 0.85 per cent saline solution, was administered by drip (60 to 80 drops/min.) or, with the smaller volumes (30 to 50 ml.), infused with a syringe at the rate of about 10 ml./min. Table I shows that plasma concentrations of 3 to 30  $\mu\text{g./ml.}$  were maintained for six to eight hours in 9 patients after a single intravenous administration of 250 to 500 mg. After 12 hours little or no antibiotic was detectable in the plasma.

## TOXICITY

Six patients developed a maculopapular rash; 4 of them had received the drug orally, 2 intravenously. Four other patients developed loose stools after oral therapy. In all instances, both rash and diarrhea subsided within 24 hours after discontinuance of therapy.

Gastrointestinal reactions were not observed in patients who received the drug intravenously. Intravenous infusions were well tolerated, and the same vein could be used repeatedly for infusion without undergoing thrombotic occlusion. Escape of a solution of novobiocin into perivenous tissues did not produce a local reaction.

No other systemic reactions were encountered in any of the patients receiving oral or intravenous novobiocin. Renal injury was not observed, since none of the patients developed albuminuria, hematuria, azotemia, or oliguria in the course of therapy, and the blood urea nitrogen was not increased in the course of therapy in patients with renal insufficiency. Anemia, leukopenia, thrombocytopenia, or agranulocytosis did not develop. These data are in agreement with those of other investigators.<sup>2-4</sup>

\* The trade name of Merck Sharpe & Dohme for novobiocin is Cathomycin. The drug used in this study was supplied as novobiocin and as crystalline monosodium novobiocin.

TABLE I  
Novobiocin Serum Levels at Various Intervals Following Intravenous  
Administration of 250 and 500 mg.\*

Patient no.	Dose, mg.	Free acid, $\mu\text{g.}/\text{ml.}$ at hours						
		1	2	4	6	8	12	24
1	250	14	9		0	0	0	0
2	250	33	20	11	2			0
3	500	56	23	21	12		2	0
4	500	30	27	10	5	2	0	0
5	500	23	11.8	7.9	4.6	2.4		
6	500	31	20.6	10.4	5.9	2.9		
7	500	18.4	11.0	3.6				
8	500	20.6	11.6	7.0	4.0	2.0		
9	500	—	16.1	7.2	3	2.11		
10	500	26.1	16.5	10.0		4.2		
11	500	20.9	15.8	11.0		5.9		

\* Sensitive bacterial strains were inhibited by in vitro novobiocin concentrations of 12.5  $\mu\text{g.}/\text{ml.}$  or less. Resistant strains required 25  $\mu\text{g.}/\text{ml.}$  or more for growth inhibition.

#### CLINICAL OBSERVATIONS

*Infections Complicating Peripheral Vascular Disease.* Thirteen patients in this category received novobiocin (table II). Hemolytic *Staphylococcus aureus*, coagulase-positive, was cultured in 10, *Proteus morgagni* and beta-hemolytic *Streptococcus* in 1, and *Pseudomonas aeruginosa*, *Escherichia coli*, and nonhemolytic *Streptococcus* in 1. In the remaining patient, no culture was obtained. Of the 10 strains of *Staph. aureus* isolated, 7 were tested and found to be sensitive to novobiocin (growth inhibited in vitro by less than 10  $\mu\text{g.}/\text{ml.}$ ).

Six of the 13 infections healed rapidly, five showed slow but satisfactory healing, and two were failures. Of the 11 who responded, prior antibiotic therapy had failed in 6, and 5 received novobiocin from the beginning (table III). These patients received 1 Gm./day for 13 to 60 days in divided doses without toxic effects. In patients who responded the infection was promptly controlled. Systemic and local signs of the infection subsided, in spite of an environment of devitalized tissue with impaired arterial circulation.

The two failures were patients with total blockade of the peripheral arterial circulation, and amputation was required in the face of infections due to staphylococcal strains sensitive in vitro to novobiocin.

*Soft-Tissue Infections.* PRIMARY INFECTIONS. Of the 55 patients in this group, 27 had spreading cellulitis with or without suppuration, and 28 had localized suppuration (furuncle, carbuncle, and abscess). In all instances there was associated leukocytosis and fever. Twenty-seven patients had failed to respond to prior therapy (penicillin alone in 12, and combined with streptomycin in 2, with erythromycin in 3, with chloramphenicol in 2, with streptomycin and tetracycline in 1, with tetracycline in 2, with tetracycline and chloramphenicol in 1, with erythromycin and chloramphenicol in 1, and 3 other patients had received five to six different antibiotics prior to novobiocin).

Hemolytic *Staph. aureus*, coagulase-positive was cultured from 30 patients. *Staphylococcus albus*, coagulase-negative, was cultured in 5 and *Bacillus subtilis* in 1. In 19 patients cultures were not obtainable. Twenty-two of the 30 strains of *Staph. aureus* isolated were tested for novobiocin sensitivity in vitro by a twofold tube dilution method, and 21 strains were found to be sensitive, i.e., bacterial growth was inhibited by a concentration of 12  $\mu\text{g.}/\text{ml.}$  or less. The remaining strain

was inhibited by 25  $\mu\text{g./ml.}$  All hemolytic staphylococcal strains were found to be resistant in vitro to penicillin, requiring concentrations of 5 to 10 units/ml. or higher for inhibition of growth in vitro. Those patients who received penicillin failed to respond.

The results of therapy were considered good if there was a prompt (48 to 72 hours) response with rapid and progressive improvement. In such instances there was a prompt defervescence, all local and systemic evidence of infection subsided, drainage from the infected wound decreased and frequently became sterile, and the wound rapidly healed. The results were considered poor or doubtful if the patient might have been expected to do just as well without the drug, if other measures which may have been responsible for the improvement were undertaken along with novobiocin therapy, or if there was no improvement. The results were good in 52 of the 55 patients in this category and poor in the remaining 3. Of the 52 patients who responded to novobiocin, 27 had failed to respond to prior therapy with other antibiotics. Of the three failures, 1 patient with cellulitis of the face and staphylococcal septicemia was preterminal with uremia and pulmonary edema and died, although the staphylococcal strain was inhibited in vitro by less than 12  $\mu\text{g./ml.}$  of novobiocin. The second failure was in a 14 day old infant who succumbed to an overwhelming staphylococcal septicemia, in spite of massive therapy with penicillin, streptomycin, novobiocin, erythromycin, and chloramphenicol, to all of which the bacterial strain was sensitive (growth inhibited in vitro by less than 6.25  $\mu\text{g./ml.}$  of novobiocin). The third failure was in a 40 year old woman with a pelvic abscess due to *E. coli* secondary to a ruptured appendix. She improved slowly only after drainage of the abscess.

**SECONDARY INFECTIONS.** Of the 27 patients in this group, 24 had postoperative wound infections and 3 had infections complicating burns. *Staph. aureus*, coagulase-positive, was cultured in 16, *B. subtilis* in 1, *E. coli* in 2, *Aerobacter aerogenes* in 2, beta-hemolytic *Streptococcus* and *Escherichia intermedium* in 1 each. In 4 cases no cultures were obtained.

Nineteen of the 24 patients (79 per cent) with postoperative infections responded to novobiocin. Five of them received the drug from the beginning and 14 had prior unsuccessful therapy with other antibiotics: penicillin in 3; penicillin and streptomycin in 4; tetracycline in 2; penicillin, streptomycin and tetracycline in 2; penicillin, streptomycin, erythromycin and sulfonamides in 1; erythromycin in 1; and penicillin, streptomycin, and chloramphenicol in 1.

The results were poor in 5 patients. One patient with portal cirrhosis, urinary tract infection, and carcinoma of the thyroid developed staphylococcal wound infection following partial resection of the thyroid carcinoma wherein considerable tumor was left behind. The bacterial strain was only moderately sensitive to novobiocin (growth inhibited in vitro by 12 to 25  $\mu\text{g./ml.}$ ). His wound continued to suppurate in spite of intensive local and systemic therapy with novobiocin as well as other antibiotics. He developed staphylococcal septicemia and died. Two others had microorganisms (*E. coli* and *A. aerogenes*) that were resistant to novobiocin (growth not inhibited in vitro by concentration greater than 50  $\mu\text{g./ml.}$ ) from the beginning. The fourth patient developed pneumonia and extensive wound infections due to a novobiocin-sensitive (growth inhibited in vitro by less than 12  $\mu\text{g./ml.}$ ) strain of *Staph. aureus* following ligations and excision of varicose veins, and both the wound infection and pneumonia failed to respond. He improved slowly after many weeks of hospitalization. The fifth patient had a postappendectomy wound infection due to *E. coli* that responded to appropriate drainage.

TABLE II  
Effect of Novobiocin in Infections Complicating Peripheral Vascular Disease

Patient no.	Diagnosis	Cultures	In vitro sensitivity	Days of therapy	Prior therapy	Bact.	Clinical result	Comment
No Healing								
1	Buerger's disease, cellulitis toe, subungual abscess	<i>Staph. aureus</i> , coagulase-positive	Not tested	16	None	No change	No change	Improved following incision and drainage
2	Occlusive arteriosclerotic peripheral vascular disease, ulcer and osteomyelitis toe	<i>Staph. aureus</i> , coagulase-positive	Sensitive	25	None	No change	No change	Amputation required
Slow But Satisfactory Healing								
3	Occlusive arteriosclerotic peripheral vascular disease, diabetes, gangrene of toe, cellulitis foot and leg	<i>Staph. aureus</i> , coagulase-positive	Not tested	13	Erythromycin	—	Infection subsided	Amputation required
4	Occlusive arteriosclerotic peripheral vascular disease, diabetes, gangrene toe	<i>Staph. aureus</i> , coagulase-positive	Sensitive	24	None	—	Infection controlled	Transmetatarsal amputation with rapid healing
5	Occlusive arteriosclerotic peripheral vascular disease, gangrene toe, cellulitis leg	<i>Staph. aureus</i> , coagulase-positive	Not tested	11	Penicillin	—	Sepsis controlled followed by amputation	Amputation of lower leg with rapid healing
6	Occlusive arteriosclerotic peripheral vascular disease, diabetes, gangrene toe	<i>Staph. aureus</i> , coagulase-positive	Sensitive	13	Penicillin, erythromycin, nitrofurantoin	—	Slow progressive healing after appropriate amputation	
7	Occlusive arteriosclerotic peripheral vascular disease, gangrene both feet and legs	$\beta$ -hemolytic <i>Streptococcus</i> , <i>P. morgani</i>	Sensitive	29	Penicillin, streptomycin, chloramphenicol	—	Sepsis promptly controlled after amputation	

Table II Continued on Page 391

TABLE II (Continued)  
Effect of Novobiocin in Infections Complicating Peripheral Vascular Disease

Patient no.	Diagnosis	Cultures	In vitro sensitivity	Days of therapy	Prior therapy	Bact.	Clinical result	Comment
Rapid Healing								
8	Occlusive arteriosclerotic peripheral vascular disease, diabetes, ulcer and cellulitis toe	<i>Staph. aureus</i> , coagulase-positive	Sensitive	7	Penicillin, tetracycline	—	Healed	Healed
9	Occlusive arteriosclerotic peripheral vascular disease, gangrene toe	<i>Staph. aureus</i> , enterococci	Sensitive	15	None	—	Rapid healing	Infection controlled, healed after amputation of toe
10	Occlusive arteriosclerotic peripheral vascular disease, cellulitis toe, diabetes	<i>Staph. aureus</i> , $\beta$ -hemolytic <i>Streptococcus</i> , $\beta$ -hemolytic enterococcus	Sensitive	25	None		Sterile	Healed
11	Occlusive arteriosclerotic peripheral vascular disease, cellulitis	<i>Staph. aureus</i> , <i>E. coli</i>	Sensitive Resistant	6	None		No exudate	Healed
12	Occlusive arteriosclerotic peripheral vascular disease, cellulitis and ulcer toe	No culture		8	None		No exudate	Healed
13	Occlusive arteriosclerotic peripheral vascular disease, cellulitis foot and leg, gangrene toe, sepsis stump after amputation of toe	<i>Ps. aeruginosa</i> , <i>Streptococcus</i> (nonhemolytic)	Resistant Sensitive	12	Penicillin, streptomycin, erythromycin	—	—	Healed

TABLE III  
Clinical Data in Patients with Pulmonary Infections

Patient no.	Diagnosis	Culture	In vitro sensitivity to novobiocin	Previous therapy	Days of therapy*	Bact.	Clinical result
1	Bronchopneumonia postoperative	<i>Staph. aureus</i>	Sensitive†	Penicillin, streptomycin, chloramphenicol	6	<i>Staph. albus</i>	Good
2	Bronchopneumonia postoperative	<i>Staph. aureus</i>	Sensitive	Penicillin, streptomycin	12	Sterile	Good
3	Bronchopneumonia postoperative	<i>Staph. aureus</i>	Sensitive	Tetracycline	9	Diphtheroids	Good
4	Bronchopneumonia postoperative	<i>Staph. aureus</i>	Sensitive	None	10		Good
5	Bronchopneumonia postoperative and pulmonary embolism	<i>Staph. aureus</i>	Sensitive	Penicillin, streptomycin	6	Sterile	Good
6	Bronchopneumonia postoperative	<i>Staph. aureus</i>	Sensitive	Tetracycline	8	Diphtheroids	Good
7	Bronchopneumonia postoperative	<i>Staph. aureus</i>	Sensitive	Penicillin	8	Sterile	Good
8	Pneumonitis, pulmonary embolism	<i>Staph. aureus</i>	Sensitive	Tetracycline	7		Good
9	Lobar pneumonia,‡ malignant lymphoma	<i>Staph. aureus</i>	Sensitive†	Penicillin, sulfonamides	5		Poor
10	Pneumonia,‡ diabetes, burns (3rd degree, 20 per cent)	<i>Staph. aureus</i>	Resistant	Penicillin, neomycin	3	Same	Poor
11	Bronchopneumonia,‡ postoperative septicemia			Penicillin, tetracycline	9		Poor
12	Lobar pneumonia,‡ septicemia, leukemia	<i>Staph. aureus</i>		None	6		Poor
13	Bronchopneumonia,‡ pulmonary tuberculosis	<i>E. coli</i> , <i>A. aerogenes</i> , <i>Myc. tuberculosis</i>	Resistant	Penicillin, streptomycin, tetracycline	7	Same	Poor
14	Bronchopneumonia postoperative	<i>A. aerogenes</i>	Sensitive	None	5	<i>Staph. aureus</i>	Poor
15	Bronchopneumonia,‡ portal cirrhosis			None	10		Poor
16	Bronchopneumonia postoperative	<i>A. aerogenes</i>		None	8	<i>Staph. aureus</i> , <i>A. aerogenes</i>	Poor

\* Dosage was 1 Gm./day, given intravenously or orally. † Inhibited in vitro by 3 µg./ml. or less. ‡ Terminal pneumonia.

TABLE IV  
*Effect of Novobiocin in Patients with Miscellaneous Surgical Infections*

Patient no.	Diagnosis	Wound culture	In vitro sensitivity	Prior therapy	Result
1	Enterocolitis	<i>Staph. aureus</i>	Sensitive	Chlortetracycline	Good
2	Urinary infection	<i>Staph. aureus</i>		None	Good
3	Urinary infection	<i>Staph. albus</i>		Tetracycline	Good
4	Urinary infection	<i>Staph. aureus</i>		Tetracycline	Good
5	Urinary infection	<i>B. proteus</i>		None	Good
6	Urinary infection	<i>B. proteus</i>		Chloramphenicol	Poor
7	Urinary infection	<i>A. aerogenes</i>		Chloramphenicol	Poor
8	Urinary infection	Enterococci		Tetracycline	Poor
9	Osteomyelitis	<i>Staph. aureus</i>	Sensitive	Tetracycline	Good
10	Osteomyelitis	<i>Staph. aureus</i>	Sensitive	Penicillin	Good
11	Osteomyelitis	<i>Staph. aureus</i>	Resistant	None	Poor
12	Osteomyelitis	<i>Staph. aureus</i>	Resistant	None	Poor
13	Septicemia	<i>Staph. aureus</i>	Sensitive	Penicillin, chloramphenicol, neomycin	Good
14	Septicemia	<i>Staph. aureus</i>	Resistant	Tetracycline	Poor
15	Septicemia and pancreatitis	$\beta$ -hemolytic enterococcus	Sensitive	Penicillin	Good
16	Pancreatitis			Penicillin, nitrofurantoin	Poor
17	Pancreatitis			None	Poor
18	Fever, unknown origin			Tetracycline	Good
19	Fever, unknown origin				Poor

Two of the 3 patients with burns, infected with *Staph. aureus*, coagulase-positive, in 2 and beta-hemolytic *Streptococcus* in 1, responded to novobiocin. The third patient with infection due to *Staph. aureus* (resistant in vitro to novobiocin as well as six other antibiotics) failed to respond.

**Pulmonary Infections.** In 16 patients with pulmonary infections (table III), hemolytic *Staph. aureus* was cultured in 11, *A. aerogenes* in 2, and *A. aerogenes*, *Mycobacterium tuberculosis*, and *E. coli* in 1. No cultures were obtained in 2 patients. Eleven patients had failed to respond to prior therapy with other antibiotics (table III). Eight of the 16 patients with infections due to hemolytic *Staph. aureus* showed a good response to novobiocin with defervescence and regression of all signs and symptoms in 48 to 72 hours.

Patients with pulmonary tuberculosis (1) and infections due to *A. aerogenes* (2) did not respond. Five other failures were in patients with advanced preterminal bronchopneumonia.

**Miscellaneous Infections.** This group included a variety of surgical infections listed in table IV. Hemolytic *Staph. aureus* was responsible for the infection in 9 of 19 patients.

Six of the 9 patients with staphylococcal infections responded to novobiocin. Five of these 6 had failed to respond to prior therapy with other antibiotics. Three patients with infections due to novobiocin-resistant strains failed to respond to this drug.

Of the other 10 patients, 4 responded to novobiocin therapy and 6 did not (table IV).

#### COMMENT

Patients with infections due to hemolytic staphylococci and other gram-positive microorganisms, including strains resistant to other antibiotics, responded to novo-

biocin therapy, given exclusively or following failure to respond to other antibiotics.

Novobiocin could be given orally or intravenously, yielding plasma concentrations of 6 to 46  $\mu\text{g.}/\text{ml.}$  on a daily dosage schedule of 1 Gm. orally or after 1 to 2 Gm. given intravenously in daily divided doses. These plasma concentrations were generally higher than in vitro bactericidal titers required to inhibit staphylococcal strains. In vitro studies indicated that most staphylococcal strains were inhibited by low concentrations of novobiocin.<sup>1,3</sup>

The clinical response correlated well with the results of the in vitro sensitivity. Sixty-one of the 78 hemolytic *Staph. aureus* strains isolated were tested for in vitro sensitivity to novobiocin. Fifty-seven strains were adjudged sensitive, i.e., inhibited by novobiocin concentrations of 12.5  $\mu\text{g.}/\text{ml.}$  or less, and four strains were resistant (25  $\mu\text{g.}/\text{ml.}$  or higher concentrations required for growth inhibitions). Patients with infections due to 51 of 57 sensitive staphylococcal strains showed a good therapeutic response to novobiocin. Patients with infections due to novobiocin-resistant strains uniformly failed to respond.

The prevalence of hospital acquired staphylococcal infections was well documented in this group of patients, since 59 of them developed their infections post-operatively or in the course of hospitalization for other reasons. Novobiocin was especially useful in the control of hospital acquired staphylococcal infections, since 49 of these 59 patients showed a good response. Furthermore, 91 per cent of the staphylococcal strains isolated from these patients were sensitive in vitro to novobiocin.

Past experience and published reports with other antibiotics as well as with novobiocin indicate that prolonged and injudicious use of novobiocin will inevitably result in the development of resistant strains. However, with appropriate surgical management of infections, including adequate drainage, débridement, removal of foreign bodies, relief of obstruction, avoidance of routine prophylaxis, and inadequate dosage forms, the useful life of novobiocin in a given environment may be extended.<sup>4</sup>

#### SUMMARY

One hundred and thirty consecutive patients with a variety of surgical infections, most of them due to strains of hemolytic staphylococci resistant to other antibiotics, received novobiocin. The drug was effective in the control of infections that had failed to respond to other antibiotics. Novobiocin could be given orally or intravenously, yielding plasma concentrations higher than the in vitro bactericidal titer required to inhibit most staphylococcal strains. The clinical response showed a close correlation with the results of in vitro sensitivity tests. The drug was well tolerated, and there were no serious systemic reactions.

#### BIBLIOGRAPHY

1. RUTENBURG, A. M.; SHAPIRO, P., AND SCHWEINBURG, F.: Novobiocin in the treatment of surgical infections due to staphylococci and other gram-positive bacteria, *New England J. Med.* 255:325-330, Aug. 16, 1956.
2. DAVID, N. A., AND BURGNER, P. R.: Clinical effectiveness and safety of novobiocin, *Antib. Med.* 2:219-229, April, 1956.
3. PULASKI, E. J., AND ISOKANE, R. K.: Novobiocin therapy of pyogenic surgical infections, *Surg., Gynec. & Obst.* 104:310-318, March, 1957.
4. FOSTER, F. P.; DEITCH, B., AND DADY, J. L.: Antibiotics in surgery. Some general principles and practical points, *Surg. Clin. North America*, pp. 811-826, June, 1957.

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With the introduction of novobiocin, in 1955, another antibiotic was added to the group that had been found effective against staphylococcal infections. Clinical reports appearing since that time have demonstrated its effectiveness in a wide variety of staphylococcal infections; however, it has proved to be clinically inadequate in some cases. With most staphylococci possessing the ability to acquire resistance to antibiotics previously proved to be useful, it seems worthwhile to attempt to evaluate the current position of novobiocin in the treatment of staphylococcal disease.

## BACTERIOLOGICAL CONSIDERATIONS

Early reports<sup>10, 17, 21, 39, 40</sup> stated that most strains of staphylococci were inhibited *in vitro* by concentrations of novobiocin of 1.0  $\mu\text{g./ml.}$  of media. In higher concentrations, the action could be demonstrated to be bactericidal. Disturbing information appeared in the report of Lin and Coriell,<sup>16</sup> who demonstrated the development of resistance in serial subculture of clinical staphylococcal isolates; emergence of resistant staphylococci has become a problem in the clinical use of novobiocin.<sup>9</sup>

During the summer of 1958, 50 strains of staphylococci were selected from clinical isolates at the Hospital of the University of Pennsylvania; these included 16 strains of the epidemic phage type 80/81. Employing a twofold tube dilution technique in brain-heart infusion broth at a *pH* of 7.4, the minimal inhibitory concentration (MIC) of novobiocin, as judged by turbidimetric techniques, was found to be less than 0.195  $\mu\text{g./ml.}$  for 26 of the 50 strains tested. There was one strain resistant to more than 100  $\mu\text{g./ml.}$ , and 16 strains were sensitive to concentrations of 25 to 50  $\mu\text{g./ml.}$  The average MIC of novobiocin for the epidemic strains of staphylococci was 20  $\mu\text{g./ml.}$ ; all but one of these strains was resistant to penicillin, streptomycin, tetracycline, and erythromycin in the routine sensitivity tests performed by the pour plate method. Only two of these epidemic strains were found sensitive to concentrations of erythromycin below those easily obtained in human serum with usual dosage schedules.

Thirteen of the 16 epidemic strains were found to be susceptible to the bactericidal action of novobiocin; the average concentration for bactericidal action was found to be 31  $\mu\text{g./ml.}$  of broth or approximately 50 per cent greater than the minimal inhibitory concentration.

Without the benefit of similar studies at the time of introduction of novobiocin, no conclusive statements can be made as to a probable decreased susceptibility; however, the relatively high concentrations required by the epidemic strains of staphylococci may explain the relatively poor clinical responses in some infections, as seen in our experience.

## PHARMACOLOGICAL CONSIDERATIONS

After oral administration of novobiocin, serum concentrations have indicated relatively prompt absorption in the fasting state and slightly slower and less com-

plete absorption in the postprandial state.<sup>4</sup> Wright and co-workers<sup>43</sup> have shown little difference between the maximum concentrations in the serum following 250 or 500 mg. doses, but the average concentrations were considerably higher and better sustained during the two to six hour interval after the larger dose; the highest average concentration after 250 mg. was 11  $\mu\text{g.}/\text{ml.}$  at one hour, as contrasted with 19  $\mu\text{g.}/\text{ml.}$  at four hours after a 500 mg. dose.

In two separate unpublished studies in this laboratory, a dose of 250 mg. sodium novobiocin in fasting healthy volunteers produced peak concentrations ranging from 7 to 28  $\mu\text{g.}/\text{ml.}$  serum. Doubling the dose (500 mg.) in one study increased the peak concentrations following a single dose to a range of 14 to 32  $\mu\text{g.}/\text{ml.}$ , while concentrations were better maintained through an interval of four hours. After repeated doses, there was evidence of mild cumulative effects at the end of 24 hours. Bioassay was carried out with a modified Kirshbaum method,<sup>15</sup> employing *Micrococcus pyogenes* var. *aureus* as the test organism.

It appears from published data that there is considerable variation between the minimum and maximum concentrations. Average results in most series<sup>14, 21, 27, 42</sup> suggest that peak concentrations in serum in excess of 25  $\mu\text{g.}/\text{ml.}$  can be obtained on repeated oral doses of 500 mg., although individual cases may show concentrations as low as 8 to 12  $\mu\text{g.}/\text{ml.}$

With parenteral administration, higher concentrations can be obtained with the intravenous route than with the intramuscular route.<sup>4</sup> In 4 patients studied in our hospital, a 500 mg. dose given intramuscularly produced average serum concentrations through the first six hours following the injection that varied from 9.5 to 7.6  $\mu\text{g.}/\text{ml.}$  serum. The individual high was 10.4  $\mu\text{g.}/\text{ml.}$  and the low, 2.7  $\mu\text{g.}/\text{ml.}$  It would appear that a vigorous dosage schedule needs to be maintained if the intramuscular route is contemplated in moderately severe or resistant infections or that the intravenous route be utilized.

The distribution of novobiocin appears somewhat limited. Spitzzy<sup>35</sup> has suggested that it is limited largely to blood volume. It does not appear to cross into the cerebrospinal fluid in any significant degree.<sup>1, 4, 17, 18, 21, 34</sup> It is excreted in the bile,<sup>18, 21</sup> but other tissues and fluids studied have failed to show concentrations comparable to those in blood.<sup>18, 21, 34</sup> Protein binding<sup>21</sup> may contribute to the problem of distribution and failure of antibacterial action.

#### CLINICAL CONSIDERATIONS

Novobiocin appears to be moderately effective against a wide variety of staphylococcal infections. In a review of 28 publications,<sup>2, 3, 5-8, 11-14, 16, 18, 20, 22-33, 37, 38, 41</sup> a total of approximately 500 infections due to staphylococci were found to have been treated with novobiocin. The clinical results were described as satisfactory to excellent in 373 patients out of 503 studied; this represented a satisfactory response in approximately 74 per cent of patients treated. This response rate is comparable to that obtained by Finland and Nichols in their review<sup>9</sup> of 750 patients with all infections treatable by novobiocin.

Poor results or therapeutic failures with novobiocin were reported in septicemia, severe pneumonias, or in surgical infections in which continuous and adequate drainage was difficult to maintain.

The experiences at the Hospital of the University of Pennsylvania with novobiocin have, in general, paralleled those reported in the literature. Because the staphylococci have acquired, to a large degree, resistance against many of the commonly

employed antibiotics, the use of novobiocin was not encouraged until recently. It has not been used promiscuously at any time since its introduction in this hospital and has been limited mainly to the treatment of staphylococcal infections. This may account in part for the fact that at present 90 to 95 per cent of all coagulase-positive staphylococci are susceptible to a pour plate sensitivity test at a concentration of 25  $\mu$ g./ml. However, the clinical results have occasionally been disappointing, in spite of an indicated susceptibility of the organism.

In most instances, two simultaneously administered antibiotics have been used recently in the management of staphylococcal infections in this hospital, in hopes of decreasing or delaying the emergence of resistant mutants. This has been based largely on the theoretical consideration that a mutant resistant to two antibiotics will emerge less frequently in the presence of two antibiotics administered concurrently than in the presence of either agent singly.<sup>16,19</sup> The committee designated for control of staphylococcal infections in this hospital has recommended that novobiocin be used primarily in conjunction with chloramphenicol; the latter agent is currently effective in inhibiting in vitro about 75 per cent of coagulase-positive strains of staphylococci. Antibiotics are not recommended in trivial infections; novobiocin is recommended mainly in hospital acquired infections or when sensitivity studies suggest its usefulness.

In a review of approximately 135 records of inpatients in this hospital, with staphylococcal infections during the months of May to August, inclusive, it was found that novobiocin was used in the management of 20 patients. There were 23 patients with minor or superficial infections for whom conservative therapy with local heat, along with simple incision and drainage in many, was adequate for controlling the infection.

Of the 20 patients treated with novobiocin in conjunction with chloramphenicol, there were satisfactory or better responses in 16 patients (80 per cent) and deaths in 3 patients. The clinical diagnoses and responses in these cases were as follows: bronchopneumonia, 2 good; wound infections, 3 good, 1 improved, 1 doubtful; furuncle and carbuncle, 2 good, 5 improved; abscess, 1 good; pyoderma, 1 good; suppurative lymphadenitis, 1 good; and septicemia, 3 failures (all died).

Several of the patients with furuncles were discharged within 48 to 72 hours after incision and drainage; the sites of infection were markedly improved after novobiocin therapy. Antibiotics were used in these cases mainly to control cellulitis and lymphangitis associated with these lesions.

The 3 patients with septicemia who died were all seriously ill patients in whom a staphylococcal bacteremia became superimposed. One patient had long-standing pyelonephritis with contracted kidneys; a second patient had been admitted with volvulus of the sigmoid colon and had acquired an infection of a cut-down site for parenteral therapy, which acted as a portal of entry for the blood stream infection; the third patient had carcinoma of the rectum, intestinal obstruction, and had experienced a blood transfusion reaction with renal shutdown prior to death. An enterococcus also was found in the blood cultures of the third patient. In each of these 3, the staphylococcal infection occurred as a terminal event in an already severe and debilitating clinical disorder. Parenteral medication was used in all 3 patients, with initial intravenous usage of novobiocin in 2 of the 3 patients. In 2 of these patients, cultures prior to death did not indicate in vitro resistance to novobiocin, in spite of the unsuccessful use of this agent for 7 and 10 days prior to death.

Clinical results with novobiocin have been satisfactory in bronchopneumonia

and lobar pneumonia, but reinforcement of the therapeutic program with such agents as bacitracin, ristocetin, or vancomycin has frequently been necessary in the past. Such staphylococcal infections have been seen in the very young, the aged, or those with severe underlying disease, such as influenza, malignant disease, or blood dyscrasias.

No serious toxicity was observed in this particular group of patients; however, outpatients receiving this agent and an inpatient, who was treated with novobiocin for an infection of other than staphylococcal origin, were observed during this same period to have developed skin rashes. Typical morbilliform eruptions have been observed after six to seven days of therapy. Mild gastrointestinal complaints were recorded, but they were not of sufficient severity to necessitate discontinuation of the drug.

Novobiocin has been found to be clinically useful in the management of current staphylococcal infections in this hospital. It has been recommended on the basis of sensitivity tests to be a part of initial therapy in the treatment of hospital acquired infections due to staphylococci, as soon as the etiological agent is suspected or identified. Patients who have acquired their infections on an outpatient status have responded generally to other antibiotic programs or conservative management; however, its use is recommended whenever sensitivity tests suggest resistance of the staphylococcal isolate to other commonly employed antibiotics. On the basis of sensitivity tests, epidemic strains (phage type 80/81) may require high tissue concentrations and thus be difficult to treat, unless vigorous parenteral therapy is used.

By discriminate use of novobiocin, resistance to it has not appeared to the same degree as noted for penicillin, streptomycin, tetracyclines, and erythromycin. Whether the combined use of this agent with chloramphenicol is justified to delay further the emergence of resistant organisms remains to be determined.

#### SUMMARY

1. Novobiocin is effective *in vitro* against most strains of staphylococci isolated from clinical infections. The concentrations required to inhibit epidemic strains have been found to be much higher than nonepidemic strains.

2. Effective serum concentrations have been obtained after oral and parenteral administration of available preparations. Although blood concentrations have been observed to be high, tissue concentrations have not been found to be comparable. It does not enter the normal cerebrospinal fluid in effective concentration. Protein binding may contribute to its lack of clinical effectiveness in some instances.

3. The clinical results with novobiocin have shown it to be effective in approximately 75 per cent of patients with staphylococcal infections. Poor results have been noted in patients with septicemia and severe pneumonia, and when adequate surgical drainage was difficult to maintain.

4. Novobiocin has been recommended primarily for the treatment of staphylococcal infections and, especially, when other easily administered antibiotics are ineffective. At this hospital, it has commonly been used in combination with chloramphenicol. Data are not available, however, to prove whether this combined regimen has delayed the emergence of novobiocin-resistant mutants. It should not be used promiscuously.

5. Mild toxicity has been noted as skin rashes, rare leukopenias, and minor

gastrointestinal irritation; the skin rashes and emergence of resistant mutants are the major limiting factors in the use of novobiocin against staphylococcal infection.

## BIBLIOGRAPHY

1. BAYNE, G. M.; STRICKLAND, S. C.; GYLFE, J. M., AND BOGER, W. P.: Novobiocin: a study of plasma and spinal fluid concentrations in man, *Antib. Med.* 2:166, 1956.
2. COLVILLE, J. M.; GALE, H. H.; COX, F., AND QUINN, E. L.: Clinical observations on the use of novobiocin in penicillin-resistant staphylococcal septicemia, *Clin. Research Proc.* 5:305, 1957.
3. COOK, E.; EASTMAN, G., AND BUNN, P.: The use of novobiocin in the management of acute pneumococcal and staphylococcal infections. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 396.
4. CORBIN, E. E., AND PRIGOT, A.: Novobiocin: absorption, diffusion, and excretion studies. A preliminary report. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 392.
5. DAVID, N. A., AND BURGNER, P. R.: Clinical effectiveness and safety of novobiocin, *Antib. Med.* 2:219, 1956.
6. DAVID, N. A.; MCCAWLEY, E. L., AND DEBOLT, W. L.: New novobiocin preparations in hospital practice. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 402.
7. FERGUSON, A. D.; TEIXEIRA, G. C., AND SCOTT, R. B.: Novobiocin in common pediatric infections, *J. Pediat.* 51:655, 1957.
8. FINDLAY, C. W., JR., AND JOHNSON, B. A.: Novobiocin: antistaphylococcal action in post-surgical diarrheas. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 202.
9. FINLAND, M., AND NICHOLS, R. L.: Novobiocin. *In: Antibiotica et Chemotherapia*, Vol. 4, Basel, Switzerland, and New York, S. Karger, 1957, p. 209.
10. FROST, B. M.; VALIANT, M. E.; MCCLELLAND, L.; SOLOTOROVSKY, M., AND CUCKLER, A.: The antimicrobial activity of cathomycin, a new antibiotic. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, p. 918.
11. FULLERTON, J. M., AND SMITH, A. J.: Rapid changes in sensitivity to streptomycin and erythromycin in staphylococcal septicaemia, *Lancet* 2:818, 1956.
12. HIGH, R. H., AND HUANG, N. N.: Novobiocin: a limited clinical and bacteriologic study of its use in infants and children with *Staphylococcus aureus* infections. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 411.
13. HOLLOWAY, W. J., AND SCOTT, E. G.: Novobiocin, a clinical and laboratory study, *Delaware State M. J.* 29:35, 1957.
14. KIRBY, W. M. M.; HUDSON, D. G., AND NOYES, W. D.: Clinical and laboratory studies of novobiocin, a new antibiotic, *A.M.A. Arch. Int. Med.* 98:1, 1956.
15. KIRSHBAUM, A.; KRAMER, J.; ARRET, B., AND WINTERMERE, D. M.: Microbial assays of novobiocin in pharmaceuticals and biologic fluids, *Antib. & Chemo.* 6:504, 1956.
16. LIN, F. K., AND CORIELL, L. L.: Novobiocin, a laboratory and clinical evaluation, *Antib. Med.* 2:268, 1956.
17. LIN, F. K., AND CORIELL, L. L.: Streptonivacin: laboratory and clinical studies in the pediatric age group. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, p. 634.
18. LUBASH, G.; VAN DER MEULEN, J.; BERNTSEN, C., JR., AND TOMPSETT, R.: Novobiocin: a laboratory investigation, *Antib. Med.* 2:233, 1956.
19. MACLEOD, C. M.: Interactions of host, microbe and chemotherapeutic agent, *Bull. New York Acad. Med.* 31:427, 1955.
20. MARTIN, R.; CHABBERT, Y., AND SUREAU, B.: Novobiocin: its experimental activity in vitro and in vivo. Its importance in severe staphylococcal infections, *Presse méd.* 64:1597, 1956.
21. MARTIN, W. J.; HEILMAN, F. R.; NICHOLS, D. R.; WELLMAN, W. E., AND GERACI, J. E.: Streptonivacin (Albamycin): a new antibiotic. Preliminary report, *Proc. Staff Meet., Mayo Clin.* 30:540, 1955.
22. MARTIN, W. J.; HEILMAN, F. R.; NICHOLS, D. R.; WELLMAN, W. E., AND GERACI, J. E.: Novobiocin for infections due to *Micrococcus pyogenes*, *J.A.M.A.* 162:1150, 1956.
23. MIDDLETON, J. E., AND SELF, J. B.: Penicillin V in staphylococcal infections, *Brit. M. J.* 2:940, 1957.
24. MILBERG, M. B.; SCHWARTZ, R. D., AND SILVERSTEIN, J. N.: Novobiocin: a preliminary report of therapeutic efficacy, *Antib. Med.* 2:286, 1956.
25. MORTON, R. F.; PRIGOT, A., AND MAYNARD, A. DE L.: The clinical evaluation of novobiocin, a new antibiotic. A preliminary report, *Antib. Med.* 2:282, 1956.
26. MULLINS, J. F., AND WILSON, C. J.: Novobiocin treatment of pyoderms, *Antib. Med.* 2:201, 1956.
27. NICHOLS, R. L., AND FINLAND, M.: Novobiocin: a limited bacteriologic and clinical study of its use in forty-five patients, *Antib. Med.* 2:241, 1956.
28. PEARSON, J. Z.; SOMBERG, A.; ROSENTHAL, I.; LEPPER, M. H.; JACKSON, G. G., AND DOWLING, H. F.: A clinical and bacteriologic evaluation of novobiocin in seventy-five patients, *A.M.A. Arch. Int. Med.* 98:273, 1956.

29. PULASKI, E. J., AND ISOKANE, R. K.: Novobiocin therapy of pyogenic surgical infections, *Surg., Gynec. & Obst.* 104:310, 1957.
30. RANTZ, L. A.; RANDALL, E.; THUM, L., AND BARKER, L. F.: The effects of vancomycin, oleandomycin, and novobiocin on staphylococci in vitro, *Antib. & Chemo.* 7:399, 1957.
31. RUTENBURG, A. M.; SHAPIRO, P., AND SCHWEINBURG, F.: Novobiocin in the treatment of surgical infections due to staphylococci and other gram-positive bacteria, *New England J. Med.* 255:325, 1956.
32. SCHIRGER, A.; MARTIN, W. J., AND NICHOLS, D. R.: Micrococcal bacteremia without endocarditis: clinical data and therapeutic considerations in 109 cases, *Ann. Int. Med.* 47:39, 1957.
33. SCHNECK, H.: Recurrent *Staphylococcus aureus* pneumonia and empyema treated with novobiocin. Review of the literature, with report of a case, *J. Pediat.* 50:570, 1957.
34. SIMON, H. J.; MCCUNE, R. M.; DINEEN, P. A. P., AND ROGERS, D. E.: Studies on novobiocin, a new antimicrobial agent, *Antib. Med.* 2:205, 1956.
35. SPITZY, K. H.: Über die Bestimmung des Verteilungsvolumens der Antibiotika im Körper am Beispiel von Penicillin und Novobiocin, Presented at European Symposium I in the Biochemistry of Antibiotics, Milan, Italy, 1956.
36. TAYLOR, R. M.; MILLER, W. L., AND BROOK, M. J. V.: Streptonivicin, a new antibiotic. V. Absorption, distribution, and excretion, *Antib. & Chemo.* 6:162, 1956.
37. TRAFTON, H. M., AND LIND, H. E.: Novobiocin in the treatment of urinary tract infections. In: *Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 420.
38. TURNBULL, R. B., JR.: Clinical recognition of post-operative micrococci (staphylococci) enteritis, *J.A.M.A.* 164:756, 1957.
39. VERWEY, W. F.; MILLER, A. K., AND WEST, M. K.: A laboratory evaluation of the chemotherapeutic properties of cathomycin. In: *Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, p. 924.
40. WALLICK, H.; HARRIS, D. A.; REAGAN, M. A.; RUGER, M., AND WOODRUFF, H. B.: Discovery and antimicrobial properties of cathomycin, a new antibiotic produced by *Streptomyces spheroides* n. sp. In: *Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, p. 909.
41. WARD, V. G., AND MEYER, O. O.: Novobiocin: a review of the literature and a report of experiences with the drug, *Wisconsin M. J.* 56:127, 1957.
42. WELCH, H.; LEWIS, C. N.; PUTNAM, L. E., AND RANDALL, W. A.: A study of the sensitizing potential of novobiocin, *AM & CT* 3:27, 1956.
43. WRIGHT, W. W.; PUTNAM, L. E., AND WELCH, H.: Novobiocin: serum concentrations and urinary excretion following oral administration in man, *Antib. Med.* 2:311, 1956.

# Comparative Studies of Oleandomycin, Triacetyloleandomycin, and Erythromycin, with a Brief Review of the Literature Concerning Oleandomycin

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The purpose of this report is to review briefly the literature concerning oleandomycin and triacetyloleandomycin;\* to present certain laboratory data comparing erythromycin with oleandomycin and triacetyloleandomycin, with particular reference to the in vitro antibacterial activity of these agents; and, finally, in view of these data, to attempt to evaluate the place of triacetyloleandomycin in the treatment of staphylococcal infections.

Oleandomycin, first described under the designation P.A. 105 by Sobin et al,<sup>1</sup> in 1954, is an antibiotic elaborated by a strain of *Streptomyces antibioticus*. The generic name was proposed and its partial structural features were subsequently announced by Els and co-workers.<sup>2</sup> Chemically, oleandomycin—a basic compound—is an entity composed of the sugars L-oleandrose and desosamine, glycosidically attached to a complex lactone nucleus, termed oleandolide. Oleandomycin has been classified as a member of the macrolide group of antibiotics.<sup>3</sup> Its approximate empirical formula is  $C_{37}H_{61}NO_{12}$ .

The range of activity of oleandomycin includes the gram-positive bacteria, mycobacteria, rickettsia, large viruses, and certain protozoa. It is not active against gram-negative organisms other than *Hemophilus influenzae* and members of the *Neisseria* and *Brucella* groups. Oleandomycin does not display cross resistance with penicillin, streptomycin, tetracycline, chloramphenicol, polymyxin B, bacitracin, novobiocin,<sup>4</sup> vancomycin,<sup>5</sup> or kanamycin.<sup>6</sup> In these respects therefore oleandomycin corresponds closely to erythromycin. It is easily differentiated from erythromycin, however, by means of paper chromatography, infrared spectra, and stability in aqueous acidic solutions.<sup>1</sup>

In vitro, oleandomycin has been found to be generally less active than erythromycin against most species and strains. Garrod and Waterworth<sup>7</sup> tested 58 strains of *Micrococcus pyogenes* and noted a minimal inhibitory concentration (MIC) of 0.5  $\mu\text{g.}/\text{ml.}$  for 54 of these strains. They stated that for these strains the usual MIC of erythromycin was 0.12  $\mu\text{g.}/\text{ml.}$  Oleandomycin thus had about one quarter of the activity of erythromycin against this species. They also tested six strains of *Streptococcus pyogenes* (group A) and six strains of *Diplococcus pneumoniae*, and their findings again indicated a degree of activity of oleandomycin inferior to that of erythromycin, the difference for both species being about eightfold. In evaluating four strains of *M. pyogenes* susceptible to 0.4  $\mu\text{g.}/\text{ml.}$  of erythromycin, Jones et al<sup>8</sup> reported the MIC of oleandomycin, carbomycin, and spiramycin to be two to eight times that of erythromycin. Petersdorf et al<sup>9</sup> noted that among 54 strains of staphylococci isolated from patients, nine strains were susceptible to 0.2  $\mu\text{g.}/\text{ml.}$ , 31 strains were susceptible to 1.0  $\mu\text{g.}/\text{ml.}$ , and 24 strains were resistant to 5  $\mu\text{g.}/\text{ml.}$  or more of erythromycin. In contrast, none was susceptible to

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\* The trade name of Wyeth Laboratories for triacetyloleandomycin is Cyclamycin.

TABLE I.

Summary of Reports of Susceptibility to Oleandomycin of Erythromycin-Resistant Clinical Isolates of *Staphylococci*

Investigators	Number of erythromycin-resistant strains tested	Per cent oleandomycin-susceptible
Ross <sup>11</sup>	22	100
Needham and Geraci <sup>10</sup>	75	72
Garrod and Waterworth <sup>7</sup>	45	66
English <sup>12</sup>	101	74
Jones et al <sup>8</sup>	—	30
Petersdorf et al <sup>9</sup>	21	52
Waisbren and Strelitzer <sup>22</sup>	154	None

0.2  $\mu\text{g.}/\text{ml.}$ , 16 of the strains were susceptible to 1  $\mu\text{g.}/\text{ml.}$ , and 47 of the strains were resistant to 5  $\mu\text{g.}/\text{ml.}$  or more of oleandomycin.

Cross resistance between erythromycin and oleandomycin was found to be essentially complete for strains of staphylococci made resistant in the laboratory to either agent.<sup>1,4,9,10</sup> On the other hand, freshly isolated field strains of staphylococci found to be resistant to one agent may or may not be resistant to the other (table I). Ross<sup>11</sup> found that of 140 strains of *Staphylococcus aureus*, isolated from ward patients at a children's hospital, 22 were resistant to erythromycin, and in each instance, the organism was "either quite sensitive or moderately sensitive" to oleandomycin. Of 75 erythromycin-resistant strains of staphylococci isolated from patients, Needham and Geraci<sup>10</sup> found that 54 (72 per cent) were susceptible to oleandomycin. In a study of 45 erythromycin-resistant strains of *M. pyogenes* from various hospitals in England, "double" resistance in 15 and "dissociated" resistance in 30 (66 per cent) was reported.<sup>7</sup> English<sup>12</sup> reported that of 101 strains of *M. pyogenes* var. *aureus* resistant to erythromycin, 74 per cent were susceptible to 3.12  $\mu\text{g.}/\text{ml.}$  or less of oleandomycin. It was also reported that 30 per cent of erythromycin-resistant strains of staphylococci were sensitive to oleandomycin.<sup>8</sup> Petersdorf et al<sup>9</sup> found that among 21 strains of staphylococci resistant to more than 5  $\mu\text{g.}/\text{ml.}$  of erythromycin, 11 strains were susceptible to oleandomycin. Waisbren and Strelitzer<sup>22</sup> reported that of 154 strains of staphylococci resistant to more than 3  $\mu\text{g.}/\text{ml.}$  of erythromycin, none was sensitive to oleandomycin. They also noted that of 31 strains found to be resistant to oleandomycin, 53 per cent were sensitive to erythromycin. Noyes et al<sup>4</sup> reported that strains of *M. pyogenes*, with either natural or induced resistance to oleandomycin, tended to be resistant to erythromycin.

Oral administration of oleandomycin has been reported, by Baadj et al,<sup>16</sup> to produce serum concentrations rarely exceeding 2.5  $\mu\text{g.}/\text{ml.}$ , after multiple doses of .5 Gm. every four hours or 1.0 Gm. every 12 hours in 11 subjects. Most values were actually much lower. Foltz<sup>17</sup> found average serum concentrations of oleandomycin of 0.15, 0.48, 0.50, and 0.21  $\mu\text{g.}/\text{ml.}$  at 1, 2, 3, and 6 hours, respectively, following a single 250 mg. dose. In his subjects, the average 24 hour urinary excretion was 8.2 per cent in 24 hours. There is little other published data regarding serum concentrations and urinary excretion of oleandomycin.

The average values for the antistreptococcal (*Streptococcus* 98) and antistaphylococcal (*Staphylococcus* 209P and 400) activity of serum induced by oral administration of oleandomycin, erythromycin, and spiramycin were observed, by Jones and Finland,<sup>13</sup> to be highest for erythromycin, lowest for spiramycin, with oleandomycin yielding intermediate values. They concluded that oleandomy-

cin (and spiramycin) were sufficiently inferior to erythromycin to indicate that their adoption for general use in the treatment of infections was unwarranted and should be discouraged. They further stated that the use of oleandomycin should be reserved strictly for the treatment of only those rare infections that were definitely proved to be caused by organisms highly susceptible to oleandomycin and resistant to other antibiotics in common use.

Shortly after the introduction of oleandomycin, reports of the combined action of oleandomycin with other antibiotics appeared. This subject has been excellently reviewed by Jones and Finland.<sup>13</sup> They concluded that mixtures, especially in fixed ratios, of erythromycin or oleandomycin with other antibiotics—particularly tetracycline—were not justified.

Two other antibiotics, carbomycin and spiramycin, have been found to possess antibacterial spectra similar to erythromycin. These agents display incomplete cross resistance to each other and to erythromycin and oleandomycin. Because of these characteristics and poor absorption from the gastrointestinal tract, carbomycin was soon found to have so little clinical activity that its use has been almost completely abandoned.<sup>13</sup> Similarly, spiramycin was found to be inferior to erythromycin both clinically (comparative study in scarlet fever<sup>14</sup>) and pharmacologically.<sup>13, 15</sup> Lepper et al<sup>15</sup> found that during a six month period in which spiramycin was employed in the treatment of hospitalized patients, there was a significant rise in the incidence of erythromycin- and spiramycin-resistant staphylococci among the hospitalized patients.

Recently triacetyloleandomycin, a chemical modification of oleandomycin prepared by the acetylation of three free hydroxyl groups in the oleandomycin molecule, has become available.<sup>18</sup> In crossover studies, the serum levels of triacetyloleandomycin were reported to be higher than those of oleandomycin phosphate when the agents were given in the same dosage. Urinary excretion was found to be much greater, and this was interpreted as indicating greater absorption. Acute and chronic toxicity studies in animals indicated a low order of toxicity.<sup>19</sup>

It was thus of interest to compare triacetyloleandomycin and erythromycin by means of crossover study, with reference to serum concentrations, serum antimicrobial activity, urinary excretion, and urine antibacterial activity.

#### MATERIALS AND METHODS

The twofold serial dilution technique was employed to determine the minimal inhibitory concentration (MIC) of oleandomycin and erythromycin for 74 strains of staphylococci isolated from hospitalized patients with active staphylococcal infections.

Crossover studies were performed with triacetyloleandomycin (250 mg. capsules) and erythromycin stearate\* (250 mg. coated tablets) in 10 normal young adults following a single oral dose of 500 and 1000 mg. of each agent, administered in rotation at three day intervals after overnight fasting. Sera were collected at 0, 1, 3, and 6 hours and stored at -20 C. until tested. All subjects collected a 24 hour urine specimen following the administration of each agent. Serum and urine concentrations of triacetyloleandomycin and erythromycin were determined by the *Sarcina lutea* cup plate method.<sup>19</sup> Results of the assays are reported in terms of oleandomycin base and erythromycin base. The antimicrobial activity of serum

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\* The trade name of Abbott Laboratories for erythromycin stearate is Erythrocin.

TABLE II

*Susceptibility to Oleandomycin and Erythromycin of 74 Strains of Staphylococci Isolated from Patients with Active Staphylococcal Infections*

MIC, 3.12 $\mu\text{g./ml.}$ or less	Number of strains		
	<i>Staphylococcus aureus</i>	<i>Staphylococcus albus</i>	Total (per cent)
Both antibiotics	12	54	66 (89)
Erythromycin only	0	4	4 (5)
Oleandomycin only	0	1	1 (1)
Neither antibiotic	0	3	3 (4)
Total	12	62	74

and urine was determined by the twofold serial dilution technique with the same test organism.

The effects of 500 mg. multiple doses of the agents were also studied by similar crossover means in a second group of 10 healthy adults. Each subject received five doses of the antibiotics at six hour intervals, and sera were collected at 1, 3, 6, and 26 hours after the initial dose. In this study, serum antistaphylococcal activity was determined. The test organism was a coagulase-positive, hemolytic *Staph. aureus* ID 71, which had been isolated from a patient with an active staphylococcal infection.

#### RESULTS

**Susceptibility Tests.** The susceptibility to oleandomycin and erythromycin of 74 strains of staphylococci isolated from patients with active staphylococcal infections is presented in table II. Eighty-nine per cent were susceptible to 3.12  $\mu\text{g./ml.}$  or less of both antibiotics, and 4 per cent were resistant to both agents. The remaining five strains exhibited "dissociated" susceptibility patterns to these two antibiotics.

Comparison of the relative susceptibility of the 66 sensitive strains is presented in figure 1. It can be seen that 56 strains were inhibited by 0.097  $\mu\text{g./ml.}$  or less of erythromycin. None of the strains was susceptible to this amount of oleandomycin. It is apparent that 0.35 to 0.79  $\mu\text{g./ml.}$  of oleandomycin were required to inhibit the majority of these organisms. Figure 2 presents a comparison of susceptibility of the individual staphylococcal strains to erythromycin and oleandomycin. It will be noted that only one of the strains was equally sensitive to both agents and that the remainder were 2 to 32 times less susceptible to oleandomycin. Most strains were eight times more resistant to oleandomycin. These results are in agreement with data published by others<sup>13,20</sup> and indicate that, on a weight

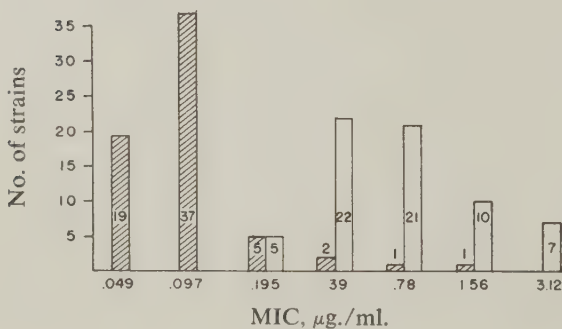
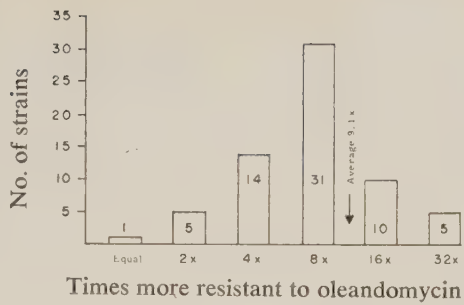


FIG. 1. Comparison of the relative susceptibility to oleandomycin and erythromycin of 66 strains of staphylococci sensitive to both agents. □, oleandomycin; ▨, erythromycin.

FIG. 2. Comparative susceptibility of 66 sensitive staphylococcal strains to oleandomycin and erythromycin.



basis, oleandomycin is biologically less active than erythromycin against most strains of staphylococci.

Erythromycin- or oleandomycin-resistant staphylococci have been relatively rarely encountered in our institution. Among the 74 strains studied, only four were resistant to erythromycin. One of these (25 per cent) was susceptible to oleandomycin. Seven strains were resistant to oleandomycin. Four (57 per cent) of these were susceptible to erythromycin. Other authors have previously reported (table I) that 0 to 100 per cent of erythromycin-resistant staphylococcal strains were susceptible to oleandomycin. There is little published data concerning the incidence of oleandomycin-resistant, erythromycin-susceptible organisms.

*Pharmacological Studies.* The results of crossover studies with erythromycin and triacetyloleandomycin in normal young adults are presented in figure 3. It can be seen that after a single 500 or 1000 mg. dose of these two agents, average antibiotic serum concentrations at 1, 3, and 6 hours were considerably higher following triacetyloleandomycin. This was most apparent at the one hour interval. At three and six hours, the antibiotic serum concentration was about three to five times higher after triacetyloleandomycin than after erythromycin.

For the performance of the first group of serum activity studies, *S. lutea* was selected as the test organism, because it was eightfold more resistant to oleandomycin than erythromycin and, in this respect, similar to the average *Micrococcus* encountered in our hospital. Also, since this organism was highly sensitive to both agents (.049 and .006  $\mu\text{g./ml.}$ , respectively), the experimental system was capable of detecting relatively smaller increments of antimicrobial activity than could be found when a more resistant test organism was employed. The results of these studies are presented in figure 4. Serum concentrations are presented as the recip-

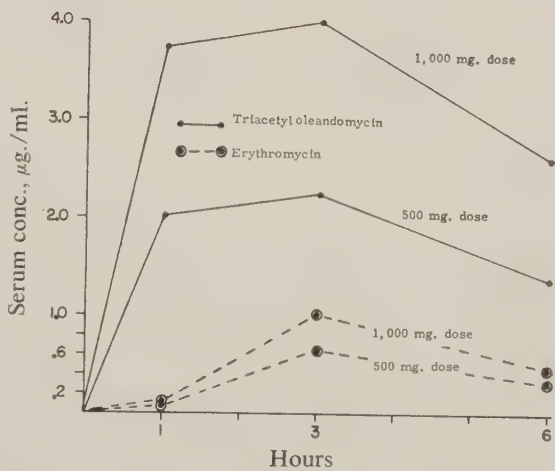


FIG. 3. Comparison of the average antibiotic serum concentrations in 10 normal adults after oral administration of a single 500 or 1000 mg. dose of triacetyloleandomycin and erythromycin. Serum concentrations measured as  $\mu\text{g./ml.}$  of erythromycin base and oleandomycin base.

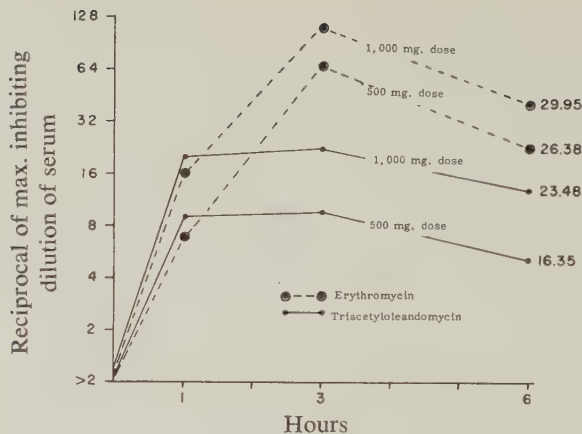


FIG. 4. Comparison of the average inhibiting dilution of the serum in 10 normal adults after oral administration of 500 or 1000 mg. single doses of triacetyloleandomycin and erythromycin. The area under each curve, expressed as the number of dilution hours, is presented to the right of each curve.

rocal of the serum dilution that inhibited growth of the test organism. It can be seen that following a single 500 or 1000 mg. dose of erythromycin or triacetyloleandomycin, the average inhibiting dilutions of the sera (antibacterial activity) were greater following triacetyloleandomycin at one hour but less at three and six hours. The area under the curves, representing the total activity of serum for the six hour period and expressed as the number of dilution hours, was greater for erythromycin than triacetyloleandomycin following the 500 or 1000 mg. dose.

The results of the multiple dose crossover study, in which serum antistaphylococcal activity was measured, are presented in figure 5. Since the *Staphylococcus* utilized as the test organism for these studies was less susceptible (MIC of oleandomycin and of erythromycin were 0.195 and 0.049  $\mu\text{g.}/\text{ml.}$ , respectively) to both antimicrobial agents than *S. lutea*, which had been employed in the previous assays, proportionately lower levels of serum activity were found for both antibiotics. In all other respects the results were similar to those observed in the previous studies.

The antimicrobial activity of serum obtained two hours after the fifth dose of each antibiotic was greater after erythromycin than that observed following similar multiple doses of triacetyloleandomycin. These results are similar to those of Reisch and co-workers,<sup>20</sup> who determined serum antistaphylococcal activity for erythromycin and triacetyloleandomycin in a similar, multiple dose crossover study. Calculated from their data, the average inhibitory dilution of serum was somewhat higher after erythromycin, i.e., 1:38.4 compared to 1:27.5 for triacetyloleandomycin.

Figure 6 compares the average 24 hour urinary excretion of the two antibiotics following a single 500 or 1000 mg. dose and indicates that approximately 18 per cent of the triacetyloleandomycin and about 1 per cent of the erythromycin was excreted during this time interval.

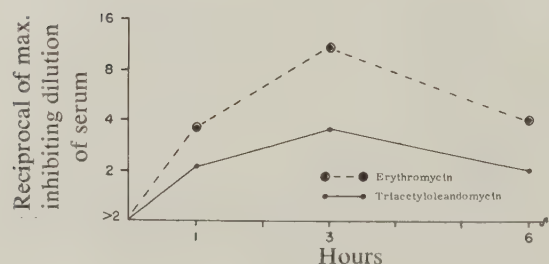
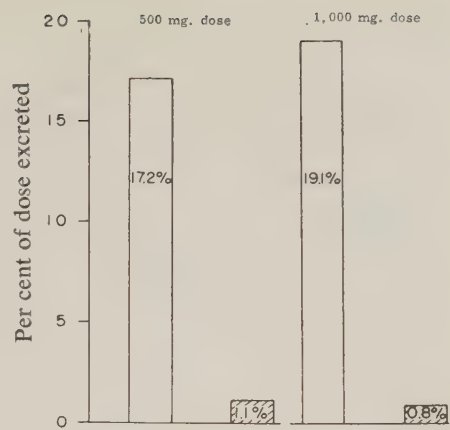


FIG. 5. Comparison of the antistaphylococcal activity of serum in 10 normal adults after oral administration of multiple 500 mg. doses of triacetyloleandomycin and erythromycin every six hours. The *Staphylococcus* test organism was resistant to penicillin, streptomycin, and tetracycline. Total dilution hours after initial dose: triacetyloleandomycin, 8.3; erythromycin, 14.1.

FIG. 6. Comparison of the average 24 hour urinary excretion of triacetyloleandomycin and erythromycin after the oral administration of a 500 or 1000 mg. single dose. □, triacetyloleandomycin; ▨, erythromycin.



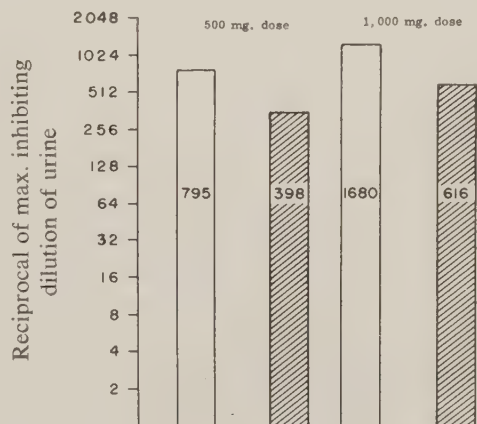
The antibacterial activity of urine, assayed with *S. lutea* as the test organism and expressed as the reciprocal of the maximal inhibiting dilution, is presented in figure 7. It can be seen that the antimicrobial activity of the urine following triacetyloleandomycin was approximately twice as great as that observed following a comparable dose of erythromycin.

Three individuals who received triacetyloleandomycin and 4 subjects who were given erythromycin experienced mild gastrointestinal reactions, characterized by slight nausea, abdominal cramps, and watery diarrhea. One subject was dropped from the study following a 500 mg. dose of erythromycin because of the intense gastrointestinal reaction. He was not given triacetyloleandomycin.

### SUMMARY AND COMMENTS

Though chemically distinct, oleandomycin, by virtue of its antimicrobial spectrum and cross-resistance pattern, has been placed in the “erythromycin-like” group of antibiotic agents, along with carbomycin and spiramycin. Considerable evidence has been accumulated by numerous investigators to indicate that, milligram for milligram, oleandomycin is biologically less active than erythromycin against a number of test organisms, including pneumococci, streptococci, and numerous strains of staphylococci. Other studies have shown that the oral administration of oleandomycin as the base, hydrochloride, or phosphate salt, resulted in serum concentrations of the agent that were somewhat greater than those ob-

FIG. 7. Comparison of the average antibacterial activity/cu. ml. of the 24 hour urinary excretion in 10 normal adults after the oral administration of a single 500 or 1000 mg. dose of triacetyloleandomycin and erythromycin. □, triacetyloleandomycin; ▨, erythromycin.



served following ingestion of an equal amount of erythromycin but not sufficiently higher to compensate for the lesser biological activity of oleandomycin. It was further observed that staphylococci, made resistant in the laboratory to either erythromycin or oleandomycin, demonstrated complete cross resistance to the other agent.

Because of these considerations, the clinical use of oleandomycin was discouraged.

More recently, a chemical modification of oleandomycin has resulted in a compound, triacetyloleandomycin, which has been reported to possess a low order of toxicity and to be capable of producing much higher serum concentrations than either oleandomycin base or erythromycin. Theoretically, an increase in serum concentration of the agent could either compensate for or exceed its biological inferiority and thus become equivalent to or exceed the activity of erythromycin.

Other reports have indicated that while staphylococci display uniform cross resistance to erythromycin and oleandomycin when resistance is induced in vitro, naturally occurring strains of staphylococci frequently demonstrated dissociated resistance patterns. These authors have variously reported that none to 100 per cent of erythromycin-resistant strains of staphylococci were sensitive to oleandomycin. Garrod<sup>23</sup> studied erythromycin-resistant strains of staphylococci isolated in various hospitals in England, with reference to cross resistance to other members of the erythromycin group of antibiotics. He showed that cross-resistance patterns varied from hospital to hospital. Thus, in one hospital almost all staphylococcal strains displayed cross resistance to all members of the erythromycin group, while in another hospital, the majority of strains showed dissociated resistance patterns.

It is thus apparent that under certain conditions, triacetyloleandomycin might prove to be a useful antistaphylococcal agent. It would further appear that an indication of the potential clinical value of this agent might be obtained by determining whether triacetyloleandomycin is capable of producing serum antimicrobial activity equivalent to or greater than erythromycin.

Other factors of importance in such an evaluation would be the determination of the actual incidence of erythromycin- or oleandomycin-resistant staphylococci and the cross-resistance patterns of these organisms within the local geographical area.

The first part of the present study indicated that within our own geographical area, the large majority of staphylococci isolated from patients with active staphylococcal infections were sensitive to both erythromycin and oleandomycin. The majority of these sensitive strains were eightfold more sensitive to erythromycin than to oleandomycin. The latter observation is in agreement with and confirms the findings of other investigators. The number of erythromycin- or oleandomycin-resistant strains was so small that valid conclusions, with reference to the incidence of associated or dissociated resistance, could not be made. It was apparent that complete cross resistance did not occur in either erythromycin- or oleandomycin-resistant organisms. The implication of this observation would seem to be that in each instance in which either erythromycin or oleandomycin resistance is encountered, one cannot empirically select the alternate agent, but that further sensitivity testing would be necessary in order to determine whether cross resistance was present.

The second part of the present study consisted of a comparison of triacetyl-

oleandomycin and erythromycin, with reference to serum concentrations, serum antimicrobial activity, 24 hour urinary excretion, and urinary antimicrobial activity by means of crossover studies in healthy adult volunteers. Comparison of serum concentrations following triacetyloleandomycin with published figures of concentrations after a similar dose of oleandomycin base, indicated that the triacetyl salt produced considerably greater concentrations. The comparative study of erythromycin and triacetyloleandomycin indicated that triacetyloleandomycin produced higher serum antibiotic concentrations throughout the experimental period than did erythromycin. However, serum antibacterial activity studies indicated that despite the previously observed superior serum concentration of triacetyloleandomycin, the antibacterial activity of serum after ingestion of this agent was slightly less than that observed after a similar dose of erythromycin. These observations were consistent following single doses of 500 or 1000 mg. of the agents and also after multiple 500 mg. doses. Variation of the test organism did not materially alter the results of these studies. These observations, with reference to comparative serum antimicrobial activity of triacetyloleandomycin and erythromycin, are in essential agreement with those of Reisch and co-workers<sup>20</sup> and Kunin and associates.<sup>21</sup>

Urinary excretion studies indicated that there was both higher concentration and greater antimicrobial activity present in the urine after triacetyloleandomycin.

Since the true test of the clinical worth of an antimicrobial agent depends upon its effectiveness in the actual treatment of disease, determined by carefully controlled studies, it is not possible from this type of study to draw conclusions as to the real value of triacetyloleandomycin as an antistaphylococcal agent. However this type of study does permit certain limited conclusions. First, the use of either triacetyloleandomycin or erythromycin for the treatment of staphylococcal infections would seem to depend largely on the characteristics of the staphylococcal population in the particular geographical area. Second, resistance of staphylococci to either erythromycin or oleandomycin does not imply either resistance or susceptibility to the other agent, and selection of the proper agent should depend on sensitivity studies.

Third, despite its ability to produce higher serum concentrations, triacetyloleandomycin induces serum antibacterial activity which is somewhat less than that produced by an equal dose of erythromycin. (Subsequent studies, to be published, employing erythromycin propionate, have indicated that this agent is capable of producing much higher blood levels and serum antimicrobial activity than erythromycin stearate. By virtue of this apparent increase in absorption, serum antimicrobial activity following erythromycin propionate was much greater than that observed following similar doses of triacetyloleandomycin.) On this basis, it would appear that in staphylococcal infections due to organisms sensitive to both antibiotics, erythromycin is the agent of choice.

#### ACKNOWLEDGMENT

The oleandomycin and triacetyloleandomycin used in this study were generously supplied by Dr. Edward F. Roberts, Wyeth Laboratories, Inc. This clinical study was aided greatly by the helpfulness and cooperation of Mrs. Belle Rowan, R.N.

#### BIBLIOGRAPHY

1. SOBIN, B. A.; ENGLISH, A. R., AND CELMER, W. D.: P.A. 105, a new antibiotic. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, pp. 827-830.

2. ELS, H.; MURAI, K., AND CELMER, W. D.: Oleandomycin (PA 105), Chemical Characteristics, Abstract of Papers, 130th Meeting of the American Chemical Society, Sept., 1956, p. 15N.
3. CELMER, W. D.; ELS, H., AND MURAI, K.: Oleandomycin derivatives. Preparation and characterization. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 476-482.
4. NOYES, H. E.; NAGLE, S. C., JR.; SANFORD, J. P., AND ROBBINS, M. L.: Novobiocin and PA 105: in vitro and in vivo studies on effectiveness against *Micrococcus pyogenes*, *Antib. & Chemo.* 6:450-455, 1956.
5. GERACI, J. E.; HEILMAN, F. R.; NICHOLS, D. R.; WELLMAN, W. E., AND ROSS, G. T.: Some laboratory and clinical experiences with a new antibiotic, vancomycin. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, pp. 90-106.
6. GOUREVITCH, V. Z.; ROSSOMANO, T. A.; PUGLISI, T. A.; TYNDA, J. M., AND LEIN, J.: Microbiological studies with kanamycin, *Ann. New York Acad. Sc.* 76:31-41, 1958.
7. GARROD, L. P., AND WATERWORTH, P. M.: Behavior in vitro of some new antistaphylococcal antibiotics, *Brit. M. J.* 2:61-65, 1956.
8. JONES, W. F., JR.; NICHOLS, R. L., AND FINLAND, M.: Development of resistance and cross resistance in vitro to erythromycin, carbomycin, spiramycin, oleandomycin and streptogramin, *Proc. Soc. Exper. Biol. & Med.* 93:388-393, 1956.
9. PETERSDORF, R. G.; BENNETT, I. L., JR., AND ROSE, M. C.: The sensitivity of pathogenic bacteria to a series of antibiotics, *Bull. Johns Hopkins Hosp.* 100:1-13, 1957.
10. NEEDHAM, G. M., AND GERACI, J. E.: Laboratory studies of oleandomycin (Matromycin), *AM & CT* 3:334, 1956.
11. ROSS, S.: PA 105, a new antibiotic. Some clinical observations. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, pp. 600-602.
12. ENGLISH, A. R.: Oleandomycin: cross resistance studies with clinical isolates of *Micrococcus pyogenes* var. *aureus*. *In: Antibiotics Annual, 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 756-759.
13. JONES, W. F., JR., AND FINLAND, M.: Antibiotic combinations; tetracycline, erythromycin, oleandomycin and spiramycin, and combinations of tetracycline with each of the other three agents—comparison of activity in vitro and antibacterial action of blood after oral administration, *New England J. Med.* 257:481-491; 536-547, 1957.
14. MARTIN, R., et al.: Résultats des traitements comparés de la scarlatine par la spiramycin et l'érythromycine: difficulté de diagnostic des farmes benignes, *Presse méd.* 63:1615, 1955. Quoted by Jones and Finland.<sup>13</sup>
15. LEPPER, M. H.; SPIES, H. W.; KELLOW, W. F. T.; ROSENTHAL, I. M., AND PLAUT, S.: Spiramycin in the treatment of infection. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, pp. 658-666.
16. BAADJ, A. G.; CORBIN, E. E., AND PRIGOT, A.: Observations on the absorption, diffusion, and excretion of oleandomycin in human beings. A preliminary report. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, pp. 72-74.
17. FOLTZ, E. L.: Personal communication.
18. ROBERTS, E. F.: Personal communication.
19. GROVE, D. C., AND RANDALL, W. A.: *Assay Methods of Antibiotics*, A Laboratory Manual, New York, Medical Encyclopedia, Inc., 1955.
20. REISCH, A. J.; MARTIN, W. J.; NICHOLS, D. R., AND HEILMAN, F. R.: Triacetyloleandomycin and erythromycin in serum. Comparison of concentrations and antibacterial effects, *Proc. Staff Meet., Mayo Clin.* 33:187-193, 1958.
21. KUNIN, C. M.; PRYLES, C. V., AND FINLAND, M.: Antibacterial activity of serum after oral doses of triacetyloleandomycin, erythromycin, potassium penicillin V and penicillin V, *Pediatrics* 22:422-431, 1958.
22. WAISBREN, B. A., AND STRELITZER, C. L.: A five year study of the antibiotic sensitivities and cross resistances of staphylococci in a general hospital. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 350-364.
23. GARROD, L. P.: The erythromycin group of antibiotics, *Brit. M. J.* 2:27-63, 1957.

# Sensitivity of *Micrococcus pyogenes* from Burned Patients to Oleandomycin and Oleandomycin-Tetracycline Combined

## Emergence of Resistance to These Antibiotics

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Bacteriostasis of *Micrococcus pyogenes* (*Staphylococcus aureus*) by the synergistic action of oleandomycin and tetracycline combined,<sup>†</sup> as well as a manufacturer's claim that such a combination delays emergence of resistance to oleandomycin, was investigated.

### MATERIALS AND METHODS

Two hundred forty-two strains of nosocomial *M. pyogenes* cultured from 57 burned patients, chosen at random at a Burn Center, served as test strains for sensitivity determinations. These microorganisms were isolated from different burned loci of patients who, to our knowledge, had never been on a drug regimen that included oleandomycin or tetracycline. All strains tested were selected from transplants on milk agar medium on which they had produced their characteristic carotinoid pigment. These micrococci were coagulase-positive, mannite fermenters, and most of them were penicillin-resistant.

Sensitivity tests were carried out by the use of wet discs (6.5 mm. Whatman no. 2 filter paper) impregnated with antibiotic solutions and by dry discs prepared and supplied by the manufacturer. The solid medium used to observe the sensitivity reactions consisted of Trypticase soy agar with blood bank outdated human blood.

Each wet disc contained a different antibiotic of approximately 0.46  $\mu\text{g}$ . concentration. This was adduced by differential weighing, wherein the saturation weight of antibiotic was found to be 0.0023 Gm. The factor, 0.0023, was used for calculating the concentration of oleandomycin, tetracycline, and the combination of these two antibiotics in the discs as follows:  $200 \mu\text{g./ml.} \times 0.0023 = 0.46 \mu\text{g./disc}$ , or an equal concentration for the three antibiotic preparations.

Commercially prepared discs containing 2, 10, and 15  $\mu\text{g./disc}$  were used as a relative measure. The presence of a zone of bacterial growth inhibition was indicative of sensitivity to the antibiotic concentration in the disc. Inhibition by 2  $\mu\text{g}$ . of oleandomycin or less was regarded as sensitivity to this drug.

Tube dilution testing to determine acquisition of resistance to oleandomycin and oleandomycin-tetracycline was done, utilizing double strength phenol red dextrose broth. Eight *M. pyogenes* strains from different patients were selected from the 242 strains used for disc testing. These were cultured for six hours at 37 C., diluted at  $3 \times 10^{-2}$ , and added in volumes of 1 ml. serial dilutions of the appropriate antibiotic. Following overnight incubation, readings were made based on fermentation of dextrose. No attempt was made to determine the nature of the inhibition, since oleandomycin and tetracycline are known to be bacteriostatic antibiotics.

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† The trade name of Chas. Pfizer & Co. for oleandomycin is Matromycin; for tetracycline, is Tetracyclin; and for oleandomycin and tetracycline combined, is Signemycin (for each 20 mg. of activity, this combination contains 13.33 mg. of tetracycline and 6.67 mg. of oleandomycin).

TABLE I

*Sensitivity of Micrococcus pyogenes to Antibiotics in Two Different Preparations*

Antibiotic	Commercial dry discs			Wet discs		
	Total no. strains	Number sensitive	Per cent sensitive	Total no. strains	Number sensitive	Per cent sensitive
Oleandomycin	143	141	98.6	99	97	97.9
Tetracycline	Not done			99	12	12.1
Oleandomycin-tetracycline	No discs had been prepared by the manufacturer			99	93	93.9

Since it was apparent from the outset that staphylococci quickly become resistant to large amounts of oleandomycin, an unrealistic plasma level of 125  $\mu\text{g.}$  was arbitrarily designated as the *in vitro* resistance point for determining which antibiotic first demonstrated emergence of resistance at this point. Eight strains were then serially transferred until this resistance figure was achieved. This was done by initially testing the microorganisms in serial halving decrements of the antibiotic, ranging from 500  $\mu\text{g.}$  to 0.25  $\mu\text{g./ml.}$  Subculture for sequential inoculum for another series of dilutions, ranging from 500 to 0.25  $\mu\text{g./ml.}$ , was then made from the tube with the lowest concentration of antibiotic showing growth as evidenced by indicator change. This was repeated until the 125  $\mu\text{g.}$  level was reached or surpassed.

## RESULTS

One hundred forty-one of 143 strains (98.6 per cent) of *M. pyogenes* proved sensitive to 2  $\mu\text{g.}$  or less of oleandomycin contained in the commercial discs. No sensitivity to commercial tetracycline discs was assessed, since it has been established repeatedly that most microorganisms are sensitive to less than 10  $\mu\text{g.}$  of this antibiotic. At the time these tests were performed, oleandomycin-tetracycline discs had not been prepared by the manufacturer.

The wet disc testing, using one-fifth the potency of the commercial discs, disclosed that 97 of 99 (97.9 per cent) *M. pyogenes* strains of the original 143 were sensitive to oleandomycin at 0.46  $\mu\text{g./ml.}$  or less. Twelve of the 99 (12.1 per cent) were sensitive to 0.46  $\mu\text{g.}$  of tetracycline, and 93 of 99 (93.9 per cent) strains were sensitive to 0.46  $\mu\text{g.}$  of the combination. It is evident that no initial synergistic bacteriostasis was realized with the combination of oleandomycin and

TABLE II

*The In Vitro Development of Antibiotic Resistance*

<i>Micrococcus pyogenes</i> strain number	Number of sequential transfers	
	Oleandomycin	Oleandomycin-tetracycline
657	3	5
681	3	4
683	15	24
684	5	24
685	2	3
688	3	15
689	2	3
690	9	24
Av.	5.2	12.7

tetracycline. When the combination was employed, a 4 per cent drop in activity occurred.

Table I illustrates the microorganism resistance to oleandomycin and to oleandomycin-tetracycline, occurring after initial exposure to these antibiotics.

Table II shows that 5 of the 8 strains required at least 125  $\mu\text{g.}/\text{ml.}$  of oleandomycin to inhibit their growth after as few as three sequential transfers. Two other strains required five and nine transfers, respectively, and the remaining strain required a total of 15 transfers. The mean number of transfers to achieve the 125  $\mu\text{g.}$  level was 5.2. For oleandomycin and tetracycline combined, 4 strains required 15 to 24 transfers to become resistant at the 125  $\mu\text{g.}$  level, while 4 strains required three to five transfers. The mean number of transfers was 12.7. Using 125  $\mu\text{g.}/\text{ml.}$  as the in vitro resistance point, it is evident that *M. pyogenes* strains become quickly resistant to large amounts of oleandomycin.

#### DISCUSSION

From data on hand, it is apparent that while oleandomycin is initially an excellent antibiotic for combatting *M. pyogenes*, refractivity to this agent is quickly acquired, necessitating the use of an additional antibiotic to forestall the emergence of resistance. The addition of tetracycline to oleandomycin gives synergistic bacteriostasis rather than an additive effect. This is readily perceived, since the microorganisms were only 12 per cent sensitive to tetracycline alone.

The effectiveness of oleandomycin-tetracycline in delaying resistance needs further investigation. In only 2, and possibly 3, of the 8 strains tested, there is a significant difference between the number of transfers required for development of resistance to oleandomycin, as compared with the combination. In 4 strains (50 per cent) a delay of only one transfer is achieved with the combination. In two instances (strains 683 and 690), a considerable number of transfers was required to achieve the resistance level figure used for both the single and the combined drugs.

This points to the necessity for a study of the concentration level achievable in the plasma that would more pertinently decide the merit of the two drugs.

#### SUMMARY

1. Of 143 clinical strains of *Micrococcus pyogenes*, most of which were resistant to penicillin, more than 98 per cent proved sensitive to as little as 2  $\mu\text{g.}/\text{ml.}$  of oleandomycin contained in a manufacturer's disc.

2. No antibiotic synergism was displayed by use of oleandomycin-tetracycline wet discs, as compared with oleandomycin alone when tested with 99 *M. pyogenes* strains.

3. The ability of oleandomycin-tetracycline to retard the emergence of resistant variants in 3 of 8 strains, as compared with their rapid development of antibiotic resistance to oleandomycin alone, has been demonstrated.

#### BIBLIOGRAPHY

1. ENGLISH, A. R.; McBRIDE, T. J.; VAN HALSEMA, G., AND CARLOZZI, M.: Biologic studies on PA 755, a combination of tetracycline and oleandomycin with synergistic activity, *Antib. & Chemo.* 6:511, 1956.

# Correlation of Sensitivity Patterns of Antimicrobial Agents by the Disc Plate Method

## A Preliminary Study Involving 5600 Gram-Positive Cocci

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Because there are a great many variable factors that affect the sensitivity of pathogenic organisms to antimicrobial agents, it is possible to determine the relative efficacy of different agents only if they are compared in the same controlled study over a long enough period of time to eliminate temporary variations. The following 16 month study of 16 antimicrobial agents is such a controlled evaluation.

### METHOD OF STUDY

From March, 1957, through July, 1958, patterns of susceptibility to various antimicrobial agents were established for more than 5600 cultures received by the Department of Clinical Bacteriology of the University of Tennessee and City of Memphis Hospitals. The evaluation was conducted in two series: (1) 500 consecutive specimens, both gram-positive and gram-negative cocci, were cultured and tested for susceptibility to antimicrobial agents; and (2) 5600 consecutive gram-positive specimens were similarly cultured and tested. The antimicrobial agents tested were broad- and medium-spectrum antibiotics, restricted-use antibiotics, and chemotherapeutic agents. Unfortunately, because of supply problems, not every organism could be tested with each agent. Specimens from patients were routinely cultured as follows: (1) Blood specimens were inoculated into thioglycollate medium (BBL) and a combination Trypticase soy agar slant and Trypticase soy broth bottle (Casteñada technique); (2) stool specimens were inoculated onto bismuth sulfite agar (Difco), MacConkey agar (Difco), S.S. agar (Difco), and into selenite broth (BBL). If a *Micrococcus* were suspected, a blood plate was also inoculated; and (3) other specimens, such as sputum, urine, and exudates, were inoculated onto blood-enriched Trypticase agar, MacConkey agar, and thioglycollate broth.

In the entire series, the sensitivity studies were done on blood agar. This laboratory is now changing almost exclusively to Trypticase soy agar (BBL) in an effort to improve sulfonamide sensitivity results. Many physicians and technicians fail to remember that the small amount of blood in a blood agar plate contains sufficient *p*-aminobenzoic acid to inhibit sulfonamide activity. This cannot be reiterated too much. The strength of the sensitivity discs used routinely in this laboratory may be seen, according to each antibiotic in table II.

### RESULTS AND COMMENTS

As expected, gram-negative organisms were considerably more resistant to antimicrobial agents than were gram-positive organisms. As shown in table I,

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This study was supported in part by J. B. Roerig Division, Chas. Pfizer & Co., Inc.

TABLE I

*Comparative Susceptibility of Gram-Positive and Gram-Negative Organisms  
to 15 Antibiotics*

Gram-positive organisms						Gram-negative organisms				
Total			Percentage			Total			Percentage	
No.	Sensi- tive	Resist- ant	Sensi- tive	Resist- ant		No.	Sensi- tive	Resist- ant	Sensi- tive	Resist- ant
Broad-Spectrum Antibiotics										
Tetracycline	261	201	60	77	23	237	130	107	55	45
Oxytetracycline	255	185	70	73	27	236	118	118	50	50
Chlortetracycline	260	205	55	79	21	245	112	133	46	54
Chloramphenicol	254	226	28	89	11	244	150	94	62	38
Medium-Spectrum Antibiotics										
Erythromycin	261	207	54	79	21	218	21	197	10	90
Oleandomycin	250	226	24	90	10	236	15	221	3	97
Novobiocin	252	242	10	96	4	231	85	146	37	63
Penicillin	260	153	107	59	41	239	5	234	2	98
Restricted-Use Antibiotics										
Bacitracin	252	242	10	96	4	2	1	1	50	50
Polymyxin	257	29	228	11	89	242	201	41	83	17
Streptomycin	254	121	133	48	52	246	115	131	47	53
Chemotherapeutic Agents										
Nitrofurantoin	38	35	3	92	8	70	13	57	81	19
Sulfisoxazole	261	25	236	10	90	234	12	222	5	95
Sulfadiazine	259	18	241	7	93	245	18	227	7	93
Triple Sulfonamides	258	17	241	7	93	238	12	226	5	95

TABLE II

*Results of Susceptibility Tests on 5600 Gram-Positive Organisms*

Drug	Concentration, μg.	Total		Percentage	
		Sensitive	Resistant	Sensitive	Resistant
Broad-Spectrum Antibiotics					
Tetracycline	30	3573	2027	63.8	36.2
Oxytetracycline	30	3215	2238	58.0	41.0
Chlortetracycline	30	3612	1915	65.4	34.6
Chloramphenicol	30	4192	1302	76.3	23.7
Medium-Spectrum Antibiotics					
Erythromycin	15	4341	1184	78.6	21.4
Oleandomycin	15	1565	160	90.7	9.3
Novobiocin	100	2411	174	93.3	6.7
Penicillin	10*	2982	2618	53.2	46.8
Restricted-Use Antibiotics					
Bacitracin	20*	56	13	81.2	18.8
Neomycin	30	2400	970	71.2	28.8
Polymyxin	300*	711	4796	12.9	87.1
Streptomycin	100	2147	3423	38.0	62.0
Chemotherapeutic Agents					
Nitrofurantoin	100	3928	271	93.5	6.5
Sulfisoxazole	300	548	4930	10.0	90.0
Sulfadiazine	300	371	5218	6.6	93.4
Triple sulfonamides	300	404	5041	7.4	92.6

\* Concentration expressed in units.

TABLE III

Susceptibility Patterns of 5600 Gram-Positive Organisms Expressed as Percentage of Each Strain Susceptible to the Antibiotic

	Staph. <i>aureus</i> <sup>a</sup>	Staph. <i>aureus</i> <sup>†</sup>	Staph. <i>aureus</i>	β-hemolytic Strepto- coccus	D. pneu- moniae	Str. viridans	Anaerobic Strepto- coccus	Micro- coccus sp.	γ-hemolytic Strepto- coccus	Str. faecalis	Micro- aerophilic Strepto- coccus	Staph. <i>albus</i>	α-hemolytic Strepto- coccus
Broad-Spectrum Antibiotics													
Tetracycline	41	58	60	95	87	89	91	100	91	51	88	67	100
Oxytetracycline	36	51	59	95	97	89	93	75	91	46	86	67	100
Chlortetracycline	38	61	57	97	98	94	97	100	91	69	89	67	100
Chloramphenicol	64	73	66	97	95	93	96	100	96	70	84	100	100
Medium-Spectrum Antibiotics													
Erythromycin	59	79	67	89	98	94	93	86	89	82	86	100	100
Oleandomycin	88	89	94	96	99	96	83	100	92	71	71	100	100
Novobiocin	96	90	98	96	93	96	86	100	—	86	86	—	—
Penicillin	25	52	44	91	97	87	84	75	79	55	70	33	50
Restricted-Use Antibiotics													
Bacitracin	73	76	100	75	100	88	33	—	—	—	—	—	—
Neomycin	94	94	92	25	34	62	69	100	38	47	47	—	—
Polymyxin	25	28	26	4	8	6	9	75	26	10	13	33	0
Streptomycin	38	58	58	17	13	36	50	50	71	21	51	67	100
Chemotherapeutic Agents													
Nitrofurantoin	95	91	87	88	98	99	95	100	81	89	89	100	100
Sulfisoxazole	3	4	3	12	72	17	10	14	33	8	0	0	0
Sulfadiazine	1	2	3	7	63	16	6	14	23	4	0	33	0
Triple sulfonamides	0	3	2	10	65	16	6	14	26	3	1	0	0

\* Coagulase-positive.

† Coagulase-negative.

polymyxin and nitrofurantoin are the only antibiotics effective against a substantial majority of the gram-negative organisms. In contrast, in the same series 11 antimicrobial agents were effective against most gram-positive organisms. The greater efficacy of agents against gram-positive organisms is seen in more detail in tables II and III. Table III, a presentation of susceptibility patterns of 5600 gram-positive organisms, is particularly interesting because it emphasizes the arbitrariness of such designations as "broad-spectrum" and "medium-spectrum" in describing antibiotics. As shown in table III, for instance, oleandomycin—a "medium-spectrum" antibiotic—has a greater effectiveness in combating more organisms than does tetracycline—a "broad-spectrum" antibiotic. Table III also illustrates the desirability of choosing an antibiotic to meet the needs of the patient being treated. It is apparent from the table, for instance, that, on the whole, more varieties of organisms are considerably more susceptible to oleandomycin than to erythromycin.

Because of the great amount of individual variability, tables I to III should be viewed with the idea that they are only rough guides to the indicated efficacy of the antimicrobial agents. They should not be used as substitutes for individual sensitivity tests.

#### SUMMARY

A preliminary study covering a 16 month period and showing susceptibility patterns of 5600 gram-positive cocci, as determined by the disc plate method, has been presented.

A preliminary study, which covers the period from March through May, 1957, and shows susceptibility patterns of approximately 245 gram-negative organisms has also been presented. In this present form these results are misleading because of a significant number of *Pseudomonas* and *Proteus* species in the group. A report on the gram-negative organisms similar to this large series is being prepared.

Of interest in this study is the wide margin of susceptibility that oleandomycin demonstrates over erythromycin, especially in view of the recent reports on cross-resistance. No such cross-resistance patterns now exist in this locale (the mid-South).

Patterns of the sulfonamides have been presented, even though they are erroneous and misleading. We use this group to reiterate that there is no accurate method known at this time of determining true sulfonamide sensitivity. Therefore we must rely on authoritative clinical trials as a guide for therapy.

# A Clinical Evaluation of Triacetyloleandomycin in the Treatment of Infections Due to Gram-Positive Cocci

## A Preliminary Report

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Oleandomycin, an antibiotic substance elaborated by a strain of *Streptomyces antibioticus*, has, until recently, been available only in the form of a phosphate salt and has been used clinically in oral, intravenous, and, occasionally, intramuscular forms. Previously the blood levels obtained from therapeutic oral doses were disappointing, but the authors have overcome this factor by the use of a "loading oral dose" and have found this antibiotic to have significant clinical effectiveness against the gram-positive cocci and especially against the coagulase-positive *Staphylococcus aureus*. The parenteral form of oleandomycin, especially that for intravenous use, has been successfully employed by many and is now undergoing extensive evaluation in our institution. Adequate blood levels can be maintained with the intravenous form, and patients are able to tolerate high dosage over prolonged periods of time. The authors, in a review of the disc method sensitivity results of 5600 gram-positive cocci,<sup>3</sup> found that oleandomycin showed in vitro effectiveness against 91 per cent of the gram-positive cocci and 88 per cent of the coagulase-positive *Staph. aureus*. Our in vitro studies at present show a slight percentage increase over these figures, which is probably a result of our adoption of the 15  $\mu$ g. oleandomycin disc (BBL). Studies in duplicate in our laboratories, which compare disc method results with tube dilution results,<sup>2</sup> add further proof that the disc method test when done properly is quite satisfactory.

Recently, through acetylation and the development of the crystalline ester, triacetyloleandomycin,\* this antibiotic has been greatly improved. Significantly higher blood levels are now obtainable, and oral tolerance is higher.

### METHOD OF STUDY

This study was performed primarily on patients from the surgical services of John Gaston Hospital. There was random sampling of age and sex, and the only prerequisite for patient acceptance was infection involving gram-positive cocci. From our study group, only coagulase-positive *Staph. aureus*, coagulase-negative *Staph. aureus*, and beta-hemolytic *Streptococcus* were isolated. Our technique for isolation, identification, and sensitivity testing was the established routine in our laboratory.<sup>3</sup> The infections were various and ranged from pediatric pyoderma to adult breast abscesses. Doses were individualized and depended upon acuteness and severity. Adult dosages ranged from 1 to 2 Gm. daily, while pediatric dosages varied from 30 to 50 mg./Kg./day. The patients were instructed to take the medication 30 minutes before meals in order to avoid possible chelation of the antibiotic in the intestinal tract. It is our impression that a "loading oral dose" significantly affects

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This study was supported in part by the J. B. Roerig and Co. Division, Chas. Pfizer & Co., Inc.

\* The trade name of J. B. Roerig & Co. for triacetyloleandomycin is Tao.

the clinical response in many cases (see discussion). Additional therapy included incision and drainage and débridement when necessary. A number of these patients had received treatment for periods as long as 30 days prior to our investigation. Many abscesses contained iodoform and nitrofurazone gauze drains, but coagulase-positive staphylococci were cultured from the depth of the wound in all cases. Some had received penicillin and sulfonamide therapy without significant response. When applicable, the patients were instructed to soak the lesion in hot water for 20 minutes four times daily. All were cautioned to keep the wound free of contamination. Patient cooperation was excellent, with a follow-up of 99 per cent of patients. Our greatest problem was obtaining culturable material on the second visit for our correlative tube dilution evaluation of original and repeat isolates. Follow-up visits varied from three to four days after the original visit. This study is a preliminary one in which 100 patients are presented, but this will eventually include a minimum of 250 patients, and more emphasis will be placed on critical hospital cases.

An investigation of the triacetyloleandomycin resistance exhibited by the organisms associated with the study is now under way. The original isolate and successive isolates, when available, are being subjected to the tube dilution sensitivity studies.<sup>2</sup>

## RESULTS

One hundred patients have thus far been evaluated. Table I illustrates the clinical response of these patients and our criteria for evaluation of this response. Of these 100 patients, 80 were considered to show excellent response by our criteria, 18 to show good response, 1 to show fair response, and none failed to respond. "Soft stools" were described by 3 patients, but these were not considered to be of sufficient magnitude to be termed side reactions. This constituted the only finding even closely resembling a drug reaction.

Patients 10, 17, and 25 are representative of the breast infections in this series.

Patient 17 had a nonlocalized cellulitis involving tissue about the areola and responded rapidly to the antibiotic in use. The pathogenic organism here was beta-hemolytic *Streptococcus*. Patients 10 and 25, young postpartum women with severe draining breast abscesses, failed to respond to previous antimicrobial therapy. Patient 10 had three draining surgical incisions of the left breast. Two coagulase-negative strains of penicillin-resistant staphylococci were isolated from two of the incisions. A coagulase-positive *Staph. aureus*, resistant to oleandomycin and penicillin, was isolated from the third drainage incision. However, in spite of the oleandomycin-resistant isolate, this patient's recovery was dramatic. After the twelfth day, her dosage was reduced from 1 Gm. to 250 mg. daily. To this date she has had no recurrence. Three of this patient's children developed furunculosis, which rapidly responded to the antibiotic.

Those cases from which beta-hemolytic *Streptococcus* was isolated displayed the most dramatic response. Within 12 to 18 hours, using a 1 Gm. daily dose, the acute signs of inflammation had subsided. Only the severe cases of impetigo were studied. This group was next in the rapidity of clearing, as within 24 to 48 hours the infected lesions were dried up. The initial cultures from these lesions revealed the usual combination of *Staph. aureus* and beta-hemolytic *Streptococcus*.

Of the patients treated for furunculosis, those who also had adequate drainage healed the fastest of this group. The chronic shallow and deep ulcers and the deep necrotic and suppurative lesions were the slowest to respond, but manifestations of sterilization, such as cleanness and the development of a more viable appearance, were seen early in this group.

TABLE I  
*Patient Response\**

Patient no.	Diagnosis	Fair	Good	Excellent
1	Pyoderma and suppurative wound			X
2	Suppurative wound with extensive cellulitis of right foot			X
3	Infected wound, duration one month		X	
4	Postoperative wound infection			X
5	Abscess following diphtheria-pertussis-tetanus immunization			X
6	Acute inguinal lymphadenitis following incision and drainage of leg abscess		X	
7	Scalp abscess			X
8	Infected second degree burns			X
9	Surgical wound abscess		X	
10	Breast abscess			X
11	Infected laceration on heel			X
12	Recurrent multiple abscesses			X
13	Multiple skin abscesses		X	
14	Suppurative appendectomy incision			X
15	Abscess in sutured laceration			X
16	Pyoderma			X
17	Breast abscess, superficial, early			X
18	Cellulitis involving lower leg			X
19	Abscess involving puncture wound			X
20	Infected laparotomy incision			X
21	Draining abscess on back			X
22	Infected second degree burns with regional lymphadenopathy			X
23	Multiple subcutaneous abscesses in a diabetic		X	
24	Chronic leg ulcer with acute exacerbation and thrombophlebitis			X
25	Breast abscess			X
26	Draining abscess with extensive cellulitis, febrile			X
27	Suppuration with complete breakdown of surgical incision			X
28	Infection in sutured laceration			X
29	Occipital furuncle, 4 cm. diameter			X
30	Multiple subcutaneous abscesses in a diabetic		X	
31	Infected sutured laceration on head			X
32	Suppurative wound			X
33	Axillary abscess			X
34	Infected third degree burn on leg			X
35	Infection in postoperative laparotomy incision			X
36	Suppuration and cellulitis involving site of leg amputation			X
37	Suppuration within appendectomy incision			X
38	Suppuration in surgical cholecystectomy incision			X
39	Infected thyroidectomy incision			X
40	Infected wound on leg with inguinal lymphadenopathy		X	
41	Abscess of right leg		X	
42	Subcutaneous abscess on back			X
43	Facial furuncles with submental lymphadenitis			X
44	Abscess on leg			X
45	Abscess on right thigh			X
46	Pyoderma			X
47	Pyoderma following mosquito bites			X
48	Severely infected third degree chemical burn on leg			X
49	Suppuration in wound of severed Achilles tendon		X	
50	Deep infected wound on leg refractory to intensive therapy		X	
51	Abscess on right buttock with inguinal lymphadenopathy			X
52	Subcutaneous abscess on right leg following trauma			X
53	Chronic leg ulcer for six weeks with acute exacerbation		X	
54	Subcutaneous abscess with extensive cellulitis after insect bite			X
55	Recurrent abscess with involvement of entire left buttock		X	
56	Streptococcal pharyngitis			X
57	Pyoderma, extensive			X
58	Infected scrotal laceration		X	
59	Cellulitis of leg following bullet wound			X

*Table I Continued on Page 421*

TABLE I (Continued)

*Patient Response\**

Patient no.	Diagnosis	Fair	Good	Excellent
60	Submental and cervical lymphadenitis, draining			X
61	Furuncles (5) postpartum, acute			X
62	Abscess at angle of mandible with regional lymphadenitis		X	
63	Suppurative postappendectomy incision			X
64	Subcutaneous abscesses			X
65	Multiple furuncles on shoulder		X	
66	Pretibial suppurative furuncle			X
67	Infection in sutured facial laceration, possible parotid fistula		X	
68	Recurrent folliculitis and furunculosis			X
69	Right palmar abscess			X
70	Chronic pretibial ulcer, bilateral with acute exacerbation			X
71	Acute urethritis and cystitis			X
72	Infected wound, right ankle			X
73	Recurrent infection in previous incision and drainage scar for abscess			X
74	Four cm. deltoid abscess at site of previous injection, two draining abscesses, right leg			X
75	"Stitch infection" facial laceration			X
76	Streptococcal pharyngitis with systemic manifestations			X
77	Streptococcal pharyngitis, early acute			X
78	Folliculitis and furuncles			X
79	Abscess little finger, right, with extensive cellulitis of arm, epitrochlear lymphadenopathy			X
80	Acute otitis media			X
81	Axillary abscess, recurrent			X
82	Furunculosis			X
83	Furunculosis			X
84	Pyoderma			X
85	Pyoderma			X
86	Recurrent furunculosis, three months; extensive sporadic ineffective treatment		X	
87	Staphylococcal pharyngitis (resistant in vitro to oleandomycin)	X		
88	Staphylococcal acne with acute cellulitis of face			X
89	Surgical removal of sebaceous cyst followed by suppuration at incision site		X	
90	Suppuration in sutured laceration, left hand			X
91	Infected hematoma of right ankle			X
92	Furunculosis			X
93	Furunculosis			X
94	Severe acne, long standing with periodic cellulitis			X
95	Furunculosis and impetigo			X
96	Furunculosis		X	
97	Acute pelvic inflammatory disease and endometritis, postabortive			X
98	Multiple facial lacerations, infected			X
99	Impetigo, severe			X
100	Severe staphylococcal acne and recurrent furuncles			X

\* Fair = prolonged response to normal or increased dosage; a variable resulting from (1) diffusibility of antibiotic at site of infection; (2) discrepancy between in vivo response and in vitro indications; and (3) patient factor, i.e., degree to which the patient follows the advice of the physician; Good = rapid remission of symptoms, i.e., diminution of cardinal signs of inflammation within 24 to 72 hours, followed in three to four days by disappearance of activity of the lesion; and Excellent = rapid remission of symptoms, i.e., meaning diminution of cardinal signs of inflammation and their manifestations within 12 to 24 hours, with marked resolution of site of inflammation within 96 hours. This does not and should not preclude sterility of lesion. There were no poor reactions and no side reactions in the entire study.

Those study patients diagnosed as acne cases have been under treatment for four to eight months, and each has responded to reinstitution of triacetyloleandomycin therapy after recurrence as a result of withdrawal of the antibiotic. Repeated cul-

tures of the lesions along with periodic throat cultures have failed to reveal any resistant strains of bacteria.

DISCUSSION

Table II presents the drug sensitivity pattern of 128 gram-positive isolates from these patients. When one compares the sensitivity pattern of the coagulase-positive *Staph. aureus* of table I with that of similar nature in a group of 5600 gram-positive cocci,<sup>3</sup> a number of observations can be made: (1) This coagulase-positive group shows a higher degree of sensitivity to the broad-spectrum antibiotic group, ranging from a 2 per cent increase for chloramphenicol to a 12 per cent increase for tetracycline. (2) Within the medium-spectrum group, this same comparison shows erythromycin to be 1 per cent higher, while oleandomycin presents an 11 per cent increase. Though triacetyloleandomycin now shows in this study a 99 per cent in vitro effectiveness, it is expected that, in further studies that will include a greater sampling of hospital endemic staphylococci, the per cent sensitive will closely approximate the 88 percentile of the broad survey. However, one significant conclusion that can be made is that this immediate pattern and the larger pattern<sup>3</sup> demonstrate that there does not appear to be a significant carryover of cross resistance from erythromycin to triacetyloleandomycin in this locale (the mid-South). Table

TABLE II  
*Sensitivity Patterns of Isolates Against 16 Commercially Available Antibiotics  
and Chemotherapeutic Agents*

		<i>Staphylococcus aureus</i> *		<i>Staphylococcus aureus</i> †		<i>β</i> -hemolytic <i>Streptococcus</i>	
	Conc., µg.	No. isolates	Per cent sensitive	No. isolates	Per cent sensitive	No. isolates	Per cent sensitive
Broad-spectrum antibiotics							
Tetracycline	30	83	48	34	71	11	100
Oxytetracycline	30	83	48	34	71	11	100
Chlortetracycline	30	83	48	34	71	11	100
Chloramphenicol	30	78	66	34	82	11	100
Medium-spectrum antibiotics							
Erythromycin	15	83	58	34	71	11	91
Oleandomycin	15	83	99	34	96	11	100
Novobiocin	100	83	100	34	100	11	91
Penicillin	10	83	47	34	68	11	100
Restricted-use antibiotics							
Neomycin	30	83	99	34	100	11	18
Polymyxin	300	83	11	34	44	11	0
Streptomycin	100	83	47	34	76	11	27
Kanamycin	30	83	94	34	100	11	73
Chemotherapeutic agents							
Nitrofurantoin	100	83	94	34	100	11	100
Sulfisoxazole‡		83	8	34	9	11	1
Sulfadiazine‡		83	0	34	0	11	0
Triple sulfonamides‡		83	1	34	2	11	0

\* Coagulase-positive, hemolytic.

† Coagulase-negative, hemolytic.

‡ In vitro sensitivity studies with sulfonamides do not fairly indicate clinical effectiveness.  
Size of disc = 300 µg.

TABLE III

*In Vitro Comparison of Sensitivity of Erythromycin and Oleandomycin*

	<i>Staphylococcus aureus</i> *		<i>Staphylococcus aureus</i> †		$\beta$ -hemolytic <i>Streptococcus</i>	
	No. isolates	Per cent sensitive	No. isolates	Per cent sensitive	No. isolates	Per cent sensitive
Oleandomycin		99		96		99
Resistant	1		1		1	
Sensitive	82		33		10	
Erythromycin		58		71		99
Resistant	35		10		1	
Sensitive	48		24		10	

\* Coagulase-positive, hemolytic.

† Coagulase-negative, hemolytic.

III more closely demonstrates the comparison of sensitivity results between triacetyloleandomycin and erythromycin. The reasons for this significant difference in the sensitivity patterns of these two drugs could include the relatively limited consumption of erythromycin in this part of the country (particularly in this hospital) and the previously limited use of triacetyloleandomycin itself. The use of the latter antibiotic is increasing, primarily because of the increased drug efficiency allowed by triacetylation. (3) Novobiocin in this present study shows patterns consistent with our larger series. (4) Findings in this series indicate that, in comparison with the large series, penicillin exhibits a 22 per cent increase in sensitivity. (5) Neomycin indicates its topical potential in both of the series. (6) Polymyxin shows a poor 11 per cent sensitivity in both this and the large series. (7) Streptomycin shows a 9 per cent increase in this study over that of the larger study. (8) Kanamycin sulfate, just recently made available to us, exhibits thus far a high sensitivity to the gram-positive cocci. Clinical trials and evaluation of its sensitivity patterns are now under way in our institution. (9) Nitrofurantoin indicates in both series a steady persistence of sensitivity and appears to be one of the more stable antimicrobials.<sup>3</sup> (10) We feel that the coagulase-negative group in this clinical study are, for the most part, pathogenic. This group has a wider margin of sensitivity, both in our study and in others. (11) The beta-hemolytic streptococci still have a high degree of sensitivity.<sup>3</sup> (12) The sulfonamide results are presented here for one purpose only: to reiterate that there is no satisfactory method of determining in vitro sensitivity patterns for this group. Thus, we must rely upon valid clinical trials as a guide for their use. Many physicians and technicians do not remember that there is sufficient *p*-aminobenzoic acid in blood, added even to Trypticase agar (BBL), to inhibit sulfonamide activity. It is the responsibility of those involved to pass on this fact to the practicing physician. In order partially to correct this problem, we are now using Trypticase agar almost entirely for sensitivity studies.

We previously mentioned our results with a "loading oral dose." By this we mean that after evaluating the patient, we sometimes chose to give an immediate adult dose of 500 mg. or an appropriate pediatric dose, followed in four to six hours by another identical dose. The dosage form after the "loading oral dose" is the standard 250 mg. capsule given four times daily.

One cannot object to this rationale when he reviews, for example, the pathological physiology of an abscess; the organisms about the periphery are in a state of "physiological activity." These are the only organisms that respond to antimicrobial therapy. In the center or core of the abscess, the organisms not dead are in a state of auto-

bacteriostasis. Thus the original dose "hits" fast and hard, while the smaller dose is sufficient to control those organisms changing from the state of bacteriostasis back to a normal metabolic phase. We have had success in controlling recurrences of abscesses by maintaining the patients on 250 to 500 mg. daily until the focus is "sterilized." In our experience with patients maintained on this low dosage for several months, we have not been able to detect the appearance of bacterial strains resistant to these dosages. We have found that this "loading dose" is effective not only for triacetyloleandomycin but also for most other antibiotics.

#### SUMMARY

Results of a 100 patient trial of triacetyloleandomycin have been presented. Ninety-nine per cent of these patients responded favorably, indicating clinical effectiveness of the drug. Side reactions were negligible. Sensitivity patterns for 128 gram-positive organisms isolated from the patients are presented, indicating the lack of a pattern of cross resistance between erythromycin and triacetyloleandomycin. Some comparison of the sensitivity pattern of this group has been made with a previous pattern established by 5600 gram-positive cocci.

#### ACKNOWLEDGMENT

We are indebted to Dr. Harwell Wilson, Professor of Surgery and Chairman of the Division of Surgery, for granting us permission to carry out these studies on his service. We are greatly appreciative of the cooperation and enthusiasm of Dr. B. F. Benton, Dr. George R. Livermore, Jr., Dr. William T. Tyson, Jr., Nurse Holifield, the Resident Surgeons, and the third year medical students in the outpatient department.

#### BIBLIOGRAPHY

1. SOBIN, B. A.; ENGLISH, A. R., AND CELMER, W. D.: P.A. 105, a new antibiotic. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, pp. 827-830.
2. FLANIGAN, C., JR., AND LEMING, B. H., JR.: A study of possible in vivo oleandomycin resistance increase in *Staphylococcus aureus*. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 977.
3. LEMING, B. H., JR., AND FLANIGAN, C., JR.: Correlation of sensitivity patterns of antimicrobial agents. A preliminary study involving 5600 gram-positive cocci. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 414.

# A Comparison of the In Vitro to In Vivo Activity of Ristocetin, Penicillin, and Erythromycin

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The relationship of in vitro to in vivo activity of antimicrobial agents is of considerable interest to those concerned with the evaluation of these agents, and a better understanding of this relationship would be of value in both laboratory and clinical research. In our laboratories a research program was undertaken on this problem in the hope of achieving two objectives: (1) the development of a procedure for evaluating the ratio of in vivo to in vitro activity; and (2) to study the ratio of several antibiotics, including ristocetin.\* We were particularly interested in ristocetin, since we had the impression from our original studies on this antibiotic that it was more active in vivo than would be expected from its in vitro activity.<sup>1,2</sup> This report presents a procedure for determining an in vitro : in vivo ratio value and data on the ratios determined with this procedure for penicillin, erythromycin,† and ristocetin with three microorganisms.

## MATERIALS AND METHODS

Ristocetin, erythromycin lactobionate, and potassium penicillin G were used in the study. For the in vitro sensitivity tests, appropriate amounts of the antibiotic stock solution in water were added to tubes containing 5 ml. of Tryptose-phosphate broth (Difco). The tubes were inoculated with 0.1 ml. of a 1:100 dilution of a 24 hour broth culture of the specific test organism. The tests were incubated at 37 C. for 48 hours.

The test organisms used for both in vitro and in vivo studies were as follows: *Staphylococcus aureus* Smith (mucin as adjuvant in vivo); *Streptococcus pyogenes* C-203; and *Diplococcus pneumoniae* ATCC 6301.

In vivo protection tests were carried out in mice. The mice used were 18 Gm. Carworth CF-1 strain. The mice were infected by the intraperitoneal inoculation of 100 to 10,000 LD<sub>50</sub> doses of the specific organism. Each antibiotic was tested simultaneously against each of the test organisms. The antibiotics were administered intramuscularly in four equal doses 1, 3, 5, and 7 hours after infection. The in vivo activity of each antibiotic was determined over a range of 0.15 to 10 mg./Kg. of antibiotic. The mice were observed for 10 days, and the 50 per cent survival dose (CD<sub>50</sub>) and 95 per cent confidence limits determined using the method of Litchfield and Wilcoxon.<sup>3</sup>

## RESULTS AND DISCUSSION

In order to compare the relative activity of each antibiotic with each of the

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\* The trade name of Abbott Laboratories for ristocetin is Spontin.

† The trade name of Abbott Laboratories for erythromycin is Erythrocin.

TABLE I

Comparative Activity of Ristocetin, Erythromycin, and Penicillin in Vitro and in Vivo  
with *Staphylococci*

Antibiotic	Bacteria tested	Ratio	
Ristocetin	<i>Staphylococcus aureus</i>	8.0	$\times 100 = 320$
		2.5	
Erythromycin	<i>Staphylococcus aureus</i>	0.19	$\times 100 = 82$
		0.23	
Penicillin G	<i>Staphylococcus aureus</i>	0.045	$\times 100 = 10$
		0.42	

bacteria studied, the following ratio was used. Relative in vitro to in vivo activity =  
Minimum Inhibitory Concentration (mg./l.)

50 Per Cent Survival Dose (CD<sub>50</sub> in mg./Kg.)  $\times 100$ .

The larger this ratio becomes, the greater the amount of in vivo activity in relation to the in vitro activity of the antibiotic.

*Staphylococci*. The ratio of in vitro and in vivo activity of ristocetin, erythromycin, and penicillin against staphylococci are summarized in table I. The ratio of in vitro to in vivo activity of ristocetin against staphylococci was much greater than was observed with erythromycin or penicillin. In comparison to erythromycin and penicillin, ristocetin has a much greater activity in staphylococcal infected mice than would be expected from the in vitro activity of this antibiotic.

*Pneumococci*. In table II are the results of a similar experiment with ristocetin, erythromycin, and penicillin with pneumococci. The ratio MIC/CD<sub>50</sub>  $\times 100$  gave a larger value for ristocetin with pneumococci than was seen with either erythromycin or penicillin. Penicillin, however, also has a greater activity in vivo against pneumococci than expected from the in vitro activity.

*Streptococci*. The results obtained with streptococci with each antibiotic are shown in table III. Ristocetin showed a relatively greater degree of activity in streptococcal infections in vivo than would be expected from the in vitro activity.

TABLE II

Comparative Activity of Ristocetin, Erythromycin, and Penicillin in Vitro and in Vivo  
with *Pneumococci*

Antibiotic	Bacteria tested	Ratio	
Ristocetin	<i>Diplococcus pneumoniae</i>	2.0	$\times 100 = 77$
		2.6	
Erythromycin	<i>Diplococcus pneumoniae</i>	0.14	$\times 100 = 2.4$
		5.8	
Penicillin G	<i>Diplococcus pneumoniae</i>	0.45	$\times 100 = 32$
		1.4	

TABLE III  
Comparative Activity of Ristocetin, Erythromycin, and Penicillin in Vitro and in Vivo  
with Streptococci

Antibiotic	Bacteria tested	Ratio	
Ristocetin	<i>Streptococcus pyogenes</i>	1.0	$\times 100 = 131$
		0.76	
Erythromycin	<i>Streptococcus pyogenes</i>	0.02	$\times 100 = 2.8$
		0.7	
Penicillin G	<i>Streptococcus pyogenes</i>	0.01	$\times 100 = 7.6$
		0.13	

#### SUMMARY

The in vitro and in vivo activity of ristocetin, erythromycin, and penicillin was determined against staphylococci, streptococci, and pneumococci. The tube dilution assay procedure was used for determining in vitro activity, while mouse protection tests were carried out to determine in vivo activity. The ratio,  $MIC/CD_{50} \times 100$ , was used for comparison of these results.

The ratio  $MIC/CD_{50} \times 100$  for ristocetin against staphylococci was 320, as compared to 82 and 10 for erythromycin and penicillin, respectively. The ratio of activity found for streptococci was 131 for ristocetin, 2.8 for erythromycin, and 7.6 for penicillin. With pneumococci, the ratios were 77 for ristocetin, 32 for penicillin, and 2.4 for erythromycin.

A comparison of the ratio values of the three antibiotics indicates that ristocetin has a significantly higher in vivo to in vitro activity than erythromycin or penicillin.

#### BIBLIOGRAPHY

1. GRUNDY, W. E.; SINCLAIR, A. C.; THERIAULT, R. J.; GOLDSTEIN, A. W.; RICKHER, C. J.; WARREN, H. B., JR.; OLIVER, T. J., AND SYLVESTER, J. C.: Ristocetin, microbiologic properties. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 687.
2. GRUNDY, W. E.; ALFORD, E. F.; RDZOK, E. J., AND SYLVESTER, J. C.: Ristocetin, the development of resistance and bactericidal activity. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 693.
3. LITCHFIELD, J. T., JR., AND WILCOXON, F.: A simplified method of evaluating dose-effect experiments, *J. Pharmacol. & Exper. Therap.* 96:99, 1949.

# Ristocetin Serum Levels in Children

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The increasing number of serious, penicillin-resistant staphylococcal infections has resulted in a clinical need for more effective antibacterial agents. One of the newer drugs is ristocetin (Spontin\*), which is effective against staphylococci as well as other gram-positive organisms.<sup>1-3</sup> The purpose of this study was to evaluate optimal dosage of the drug in children by the determination of serum and cerebrospinal fluid levels after intravenous and intramuscular administration. In the process of these studies, clinical response and toxicity were also noted.

## MATERIAL AND METHOD

Forty hospitalized patients ranging in age from 10 days to 15 years were selected for determination of serum levels from 1 to 12 hours after the administration of 12.5 or 25 mg./Kg. of ristocetin by either the intramuscular or the intravenous route. The intravenous preparation was administered intramuscularly to 8 patients, with 5 mg. cortisone added to each injection. Nine patients also had cerebrospinal fluid level determinations after administration of the drug. Of the 40 patients, 20 were treated for clinical infections (including pneumonia, cellulitis, and sepsis) from 5 to 70 days.

Blood samples were collected by venipuncture, allowed to clot, centrifuged, and the serum stored in a freezer at  $-5^{\circ}\text{C}$ . until sufficient numbers were collected for determination of ristocetin content. The specimens were then assayed with *Streptococcus pyogenes* as the test organism, using the serial twofold dilution method.

## RESULTS

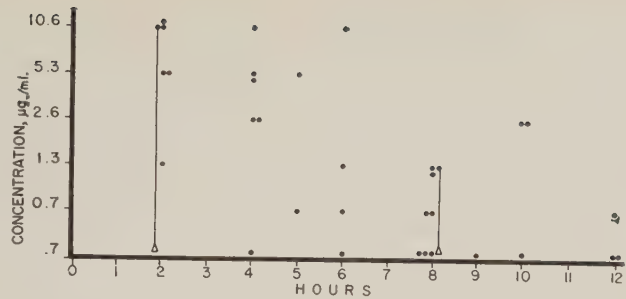
Thirty-three blood samples were taken from 2 to 12 hours after rapid intravenous administration of 12.5 mg./Kg. of ristocetin. As noted in figure 1, the ristocetin content of the serum after two hours varied from 1.3 to 10.6  $\mu\text{g./ml}$ . Thereafter there was a gradual decrease so that by 12 hours after administration, serum levels were 0.7  $\mu\text{g./ml}$ . or less. Thirty-one blood samples were collected in a like manner after an intravenous injection of 25 mg./Kg., and as noted in figure 2, the ristocetin content of the serum after one hour was 21.2  $\mu\text{g./ml}$ . The content gradually decreased, and by 12 hours after administration, serum levels ranged from less than 0.7 to 10.6  $\mu\text{g./ml}$ . Seventeen samples of blood were obtained from 2 to 12 hours after a dose of 25 mg./Kg. was given intramuscularly. Serum content as noted in figure 3 ranged from 5.3 to 21.2  $\mu\text{g./ml}$ . after two hours and remained elevated in the range of 2.6 to 10.6  $\mu\text{g./ml}$ . even after 12 hours. Blood samples were also obtained from 6 patients who received 50 mg./Kg./day by continuous intravenous drip. The serum content remained between 2.6 to 21.1  $\mu\text{g./ml}$ . during the infusion.

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Supported by a grant from Abbott Laboratories.

\* The trade name of Abbott Laboratories for ristocetin is Spontin.

FIG. 1. Ristocetin serum and cerebrospinal fluid content following a single intravenous injection of 12.5 mg./Kg. of body weight are illustrated. •, serum levels; Δ, cerebrospinal fluid levels.



In addition to the serum level determinations, simultaneous cerebrospinal fluid level determinations were accomplished on 9 patients without meningitis. As seen in figures 1, 2, and 3, the spinal fluid content of ristocetin remained less than 0.7 µg./ml. regardless of the dosage or route of administration. Nor did the duration of elapsed time after administration or the serum level at the time the cerebrospinal fluid was collected have any effect on the cerebrospinal fluid ristocetin content. Nearly all of the 20 patients treated with ristocetin either intramuscularly or intravenously for a variety of illnesses showed a good clinical response. Because of the variety of diseases and small number of cases, no significant statistical analysis of the response to the antibiotic could be made.

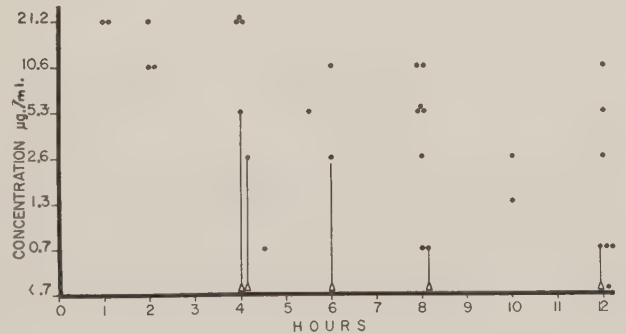
### TOXICITY

There was a minimum of side reactions in the total group of 40 patients. In 1 patient a transient area of erythema developed at the site of intravenous injection associated with nausea and vomiting. In another, erythema, tenderness, and induration occurred at the site of an intramuscular injection without cortisone. None of the 8 patients given intramuscular ristocetin with added cortisone demonstrated any reaction at the site of the injections although 2 were treated for 10 and 11 days with injections every six to eight hours. Two patients developed a generalized erythematous rash, but none of the patients demonstrated any significant hematopoietic alterations as reported by other authors.<sup>4-6</sup> Thrombophlebitis was noted in 1 patient on prolonged continuous intravenous drip.

### DISCUSSION

Ristocetin is an antibiotic elaborated by a strain of *Nocardia*. It was shown by Grundy et al<sup>7</sup> that in rabbits ristocetin was found in the blood for only three hours after intravenous administration, but up to eight hours when administered intramuscularly. Hwang et al<sup>8</sup> demonstrated that there was a gradual decreasing plasma content after intravenous administration of ristocetin to dogs. We have shown that

FIG. 2. The ristocetin serum and cerebrospinal fluid content following a single intravenous injection of 25 mg./Kg. of body weight are shown. •, serum levels; Δ, cerebrospinal fluid levels.



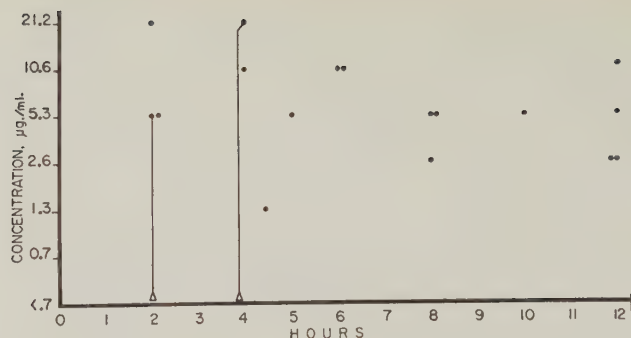


FIG. 3. Ristocetin serum and cerebrospinal fluid content following a single intramuscular injection of 25 mg./Kg. of body weight are given. •, serum levels;  $\Delta$ , cerebrospinal fluid levels.

serum ristocetin content is measurable 12 hours after administration of 25 mg./Kg. intravenously but that 12.5 mg./Kg. of intravenous ristocetin produced measurable levels only up to eight hours after administration. Correlating well with Grundy's work on rabbits, the serum content of ristocetin remains elevated even after 12 hours when administered intramuscularly.

Romansky et al<sup>2</sup> found that ristocetin was maximally active against pneumococcus, all of his 74 strains being sensitive to 3  $\mu$ g./ml. or less. All 29 strains of beta streptococci were sensitive to 5.0  $\mu$ g./ml. or less and slightly more than 70 per cent of 90 other strains of streptococci were sensitive to 5  $\mu$ g./ml. or less of ristocetin. All of 35 strains of hemolytic *Staphylococcus aureus* tested were sensitive to 5  $\mu$ g./ml. or less. These values correlated very well with the serum content and good clinical response we experienced. Although no significant antibacterial effect on gram-negative bacteria in vitro was demonstrated by Romansky, apparently in vivo a good response may be obtained, as in 1 of our patients with osteomyelitis of the right femoral head due to *Hemophilus influenzae* type B.

One patient was of particular interest, and her clinical course is outlined in figure 4. This was an 11 year old girl who developed a bacterial endocarditis due to hemolytic *Staph. aureus*. She failed to respond to large dosages of erythromycin, chloramphenicol, bacitracin, tetracycline, vancomycin, and ristocetin at 25 mg./

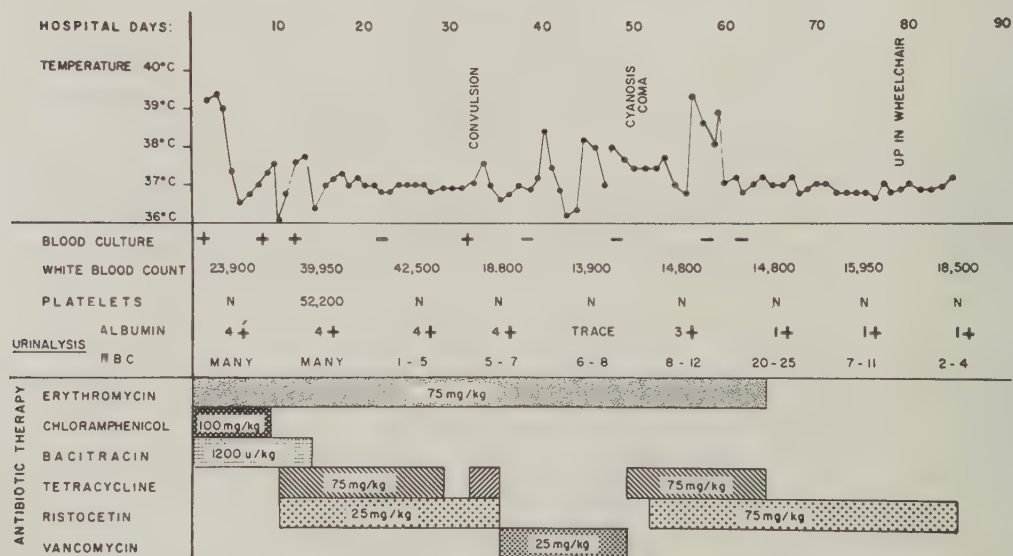


FIG. 4. The clinical course of an 11 year old girl (147284) with bacterial endocarditis due to hemolytic *Staphylococcus aureus* resistant to penicillin is given.

Kg./day dosage. However, she showed a good clinical response to a higher dosage (75 mg./Kg.) of ristocetin on which she was kept 35 days. She has remained well since discontinuation of medication.

The use of steroids to minimize the local reaction was suggested by the work of Krugman and Ebin<sup>9</sup> in which they used prednisolone with intramuscular benzathine penicillin G. Previous reports by the manufacturer had suggested that intramuscular administration was inadvisable due to local tissue reaction at the site of injection. Cortisone appeared to obviate the problem of tissue tolerance. As found by Hwang et al<sup>8</sup> in their work on dogs, there is none or only minute activity of ristocetin found in cerebrospinal fluid. In our series of 9 children, ristocetin did not occur in measurable quantities in the cerebrospinal fluid. Serious toxic reactions such as in the hematopoietic system were not observed in the dosage utilized in this study.

#### SUMMARY

1. Ristocetin was administered as a single intravenous injection of 12.5 mg./Kg. with resulting serum levels ranging from 1.3 to 10.6  $\mu$ g. after two hours with a gradual fall to a level of 0.7  $\mu$ g./ml. or less after 12 hours.

2. Administration of 25 mg./Kg. in a single intravenous injection gave an initial serum content of 10.6 to 21.2  $\mu$ g./ml., and at the end of 12 hours was between 0.7 and 10.6  $\mu$ g./ml.

3. The intravenous preparation of ristocetin was administered as a single intramuscular injection of 25 mg./Kg. The serum ristocetin content remained higher at the end of 12 hours than when a similar dosage was given intravenously. Five mg. of cortisone, added to each injection to decrease the inflammatory response, obviated the problem of local tissue tolerance.

4. Ristocetin serum content ranged from 2.6 to 21.1  $\mu$ g./ml. during continuous intravenous infusion of 50 mg./Kg./day.

5. Ristocetin did not diffuse into the cerebrospinal fluid through the uninflamed meninges.

6. For serious infections, ristocetin should be given at 12 hour intervals in a dose of 50 mg./Kg./day. For less serious infections, ristocetin may be administered at 25 to 37 mg./Kg./day dosage at eight hour intervals.

#### BIBLIOGRAPHY

1. FINLAND, M.: Antibiotics—general consideration and range of activity, *New England J. Med.* 258:86, 1958.
2. ROMANSKY, M. J.; LIMSON, B. M., AND HAWKINS, J. E.: Ristocetin: a new antibiotic—laboratory and clinical studies. Preliminary report. *In: Antibiotics Annual 1956–1957*, New York, Medical Encyclopedia, Inc., 1957, p. 706.
3. SHIDLOVSKY, B. A.; PRIGOT, A., AND TURELL, R.: The influence of ristocetin on the aerobic intestinal flora of man. *In: Antibiotics Annual 1957–1958*, New York, Medical Encyclopedia, Inc., 1958, p. 199.
4. CALVY, G. L., AND SCHUMACHER, L. R.: Ristocetin and the staphylococcus, *J.A.M.A.* 167:1585, 1958.
5. GANGAROSA, E. J.; LANDERMAN, N. S.; ROSCH, P. J., AND HERNDON, E. G.: Hematologic complications arising during ristocetin therapy, *New England J. Med.* 259:160, 1958.
6. NEWTON, R. M., AND WARD, V. G.: Leukopenia associated with ristocetin (Spontin) administration, *J.A.M.A.* 166:1959, 1958.
7. GRUNDY, W. E.; SINCLAIR, A. C.; THERIAULT, R. J.; GOLDSTEIN, A. W.; RICKHER, C. J.; WARREN, H. B., JR.; OLIVER, T. J., AND SYLVESTER, J. C.: Ristocetin, microbiologic properties. *In: Antibiotics Annual 1956–1957*, New York, Medical Encyclopedia, Inc. 1957, p. 687.
8. HWANG, K.; PRIMACK, N.; STEIN, R. J., AND RICHARDS, R. K.: Pharmacological and toxicological properties of ristocetins A and B. *In: Antibiotics Annual 1957–1958*, New York, Medical Encyclopedia, Inc., 1958, p. 163.
9. KRUGMAN, S., AND EBIN, E. V.: Improved local tolerance to benzathine penicillin G, *Pediatrics* 21:245, 1958.

# Ristocetin in the Cerebrospinal Fluid During Staphylococcal Meningitis

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Many antibiotics will traverse the so-called "blood-brain barrier" in therapeutic concentrations only in the presence of inflamed meninges (see table II). Ristocetin (Spontin\*), a new antibiotic, has been found effective against resistant staphylococci,<sup>1</sup> yet it has not previously been reported to pass into the cerebrospinal fluid. We have now been able to demonstrate its passage into the cerebrospinal fluid in a patient with staphylococcal meningitis, in significant concentration.

The components of ristocetin (ristocetins A and B) consist of four monosaccharide units each, with molecular weights in the vicinity of 4000.<sup>2</sup> This large molecular weight and size, as well as the work with laboratory animals by Hwang et al.,<sup>3</sup> suggested that this antibiotic would not penetrate normal meninges. However, in the case to be reported here, ristocetin was administered as a last resort to a moribund patient with meningitis due to a hemolytic *Staphylococcus aureus*, coagulase-positive. The patient recovered, and blood and cerebrospinal fluid analyses during the course of therapy showed that ristocetin will penetrate inflamed meninges.

## CASE REPORT

On Nov. 16, 1957, at Central Baptist Hospital (Lexington, Ky.) a 32 year old white woman was delivered, under general anesthesia, of a healthy child by low forceps. Three days later she had a fever (106 F.), headache, nuchal rigidity, and reduced reflexes on the left side with absent plantar reflex. Spinal fluid was cloudy and under slightly increased pressure. Analysis revealed 711 cells/ml. (106 polymorphonuclear leukocytes and 605 lymphocytes); globulin, 3+; and protein, 161 mg./100 ml. Culture of the fluid produced hemolytic *Staph. aureus*, coagulase-positive, as the causative organism. By testing with discs, the organism was found to be sensitive to the usual antibiotics. The tentative diagnosis was septicemia with brain abscess, accompanied by secondary meningitis.

Treatment consisted of penicillin parenterally and intrathecally, erythromycin orally and parenterally, and corticoids orally and parenterally. However, the patient's condition gradually deteriorated, and in desperation, streptomycin, chlortetracycline, and sodium sulfadiazine were added to the regimen in full dosage.

On the seventh hospital day, the patient appeared moribund. The hemoglobin had dropped to 8.3 Gm./100 ml., necessitating transfusion of whole blood. Cortisone was maintained above 200 mg. daily. On the eighth hospital day, jacksonian-type status epilepticus developed, requiring large doses of diphenylhydantoin sodium and phenobarbital sodium. Signs of right hemiplegia developed and respiration was Cheyne-Stokes; a tracheostomy was performed.

On the tenth hospital day, administration of ristocetin was initiated at a dosage of 1.0 Gm. every six hours by intravenous infusion. This was accompanied by 15 ml. of gamma globulin intramuscularly. Multiple trephining was performed in order to drain an accessible brain abscess; however, the only findings were a thick (1.5 cm.) pachymeningitis and a wet brain.

On the eleventh hospital day, there was marked improvement, which continued steadily except for suggestive clinical, roentgenographic, and electrocardiographic evidence of pulmonary embolism on the twelfth day. Monilial cystitis developed on the twenty-first day but cleared after administration of nystatin orally and vaginally.

On the thirty-second hospital day, all medications were withdrawn. The patient's only residua were a silly affect and amnesia for the entire pregnancy. Subsequently her personality has returned to normal, and parts of her memory loss are clearing.

\* The trade name of Abbott Laboratories for ristocetin is Spontin.

TABLE I  
*Ristocetin Fluid Levels during Intravenous Therapy*

Time, hr.	Serum, μg./ml.	Cerebrospinal fluid (duplicate samples), μg./ml.
24	10.24	None
48	0.64	0.32, <0.64
72	0.64	0.32, <0.32
96	10.24	0.32, 0.32

#### DISCUSSION

A review of the literature indicates that other frequently employed antibiotics pass the meninges more readily during inflammation than when the meninges are not involved. These data are summarized in table II, showing cerebrospinal fluid levels of various antibiotics as reported.

Of the antibiotics listed—erythromycin, chloramphenicol, streptomycin, dihydrostreptomycin, the tetracyclines, novobiocin, and penicillin—only chloramphenicol apparently passes with equal facility both normal and abnormal meninges (cerebrospinal fluid levels of chloramphenicol are equal to 50 per cent of serum levels). Despite this, most of these antibiotics have been used effectively for various infections of the central nervous system,<sup>6, 9-11, 16, 17, 20-24</sup> even though the spinal fluid levels obtained were less than the theoretically required therapeutic levels for some organisms. Possibly Price and Hodges<sup>25</sup> explanation regarding penicillin holds for other agents: "If the infection involves the meningeal membranes primarily, it would seem that penicillin given intravenously reaches the involved tissues through their blood supply, as it does elsewhere in the body, to exert its effect on the bacteria."

Although high doses of ristocetin were given this patient intravenously, high circulating blood levels were not regularly obtained on assay (see table I). This emphasizes the fact that individual determinations of serum and cerebrospinal fluid levels do not always reflect peak levels, but merely residual levels prior to another infusion of ristocetin. Certainly, the spinal fluid levels shown in table I do not adequately explain the therapeutic results in this case, since staphylococci generally require a minimum concentration of ristocetin equal to 4.0 μg./ml. However, ristocetin qualitatively did pass the blood-brain barrier with residual levels commensurate with those found concurrently in the serum. Perhaps the therapeutic results may be explained partly by the synergistic effect between ristocetin and gamma globulin, previously reported by Holper and co-workers.<sup>26</sup>

Staphylococcal (micrococcal) meningitis is relatively rare. Only 2 per cent of 3662 cases of purulent meningitis reported by the New York City Department of Health<sup>27</sup> for the years 1920 through 1956 were staphylococcal. In only 6 (1.7 per cent) of 354 patients with acute purulent meningitis admitted to the Los Angeles Children's Hospital<sup>28</sup> during the 10 years 1944 to 1953 was a *Micrococcus* identified, while in 15 years at the Infants' and Children's Hospital of Boston, 3.6 per cent of 864 cases of meningitis were found due to this organism.<sup>24</sup>

However infrequent, staphylococcal meningitis is always a formidable problem, increased recently by the development of strains resistant to penicillin and other antibiotics. Such resistance is emphasized by a study of 500 strains of hemolytic *Staph. aureus*, coagulase-positive, from clinical material in a hospital. Finland and Haight<sup>29</sup> found about three fourths of these strains resistant to penicillin, one fourth to chlortetracycline, and one third to oxytetracycline. Serious staphylococcal infec-

tions may be increasing. For instance, Fisher et al<sup>30</sup> studied staphylococcal pneumonia at the Johns Hopkins Hospital during the period 1942 through 1956. Of 18 cases, only 4 occurred during the first eight years, 5 in the next three years, and 9 in the last four years of the period. Their series included 3 other cases with a total mortality of 67 per cent. They concluded that infections due to drug-resistant staphylococci represent the most serious hazard. In the case reported herein, a clinical cure had not been effected by penicillin, streptomycin, erythromycin, chlor-tetracycline, and sulfadiazine despite evidence in vitro that the causative organism was sensitive to these agents.

TABLE II  
Cerebrospinal Fluid Levels and Serum Levels of Various Antibiotics

Antibiotic	No. of patients	Condition of patients and meninges	Serum levels	Cerebrospinal fluid levels
Erythromycin <sup>4, 5</sup>	14	Meninges normal; includes 5 cases of prophylaxis for prefrontal lobotomy	0.125–8.0 µg./ml.	0.0–0.250 µg./ml.
Erythromycin <sup>6</sup>	3	Pneumococcal, meningococcal, and <i>Hemophilus influenzae</i> meningitides	5–20 µg./ml.	0.3–2.50 µg./ml.
Chloramphenicol <sup>7, 8</sup>	>3	Meninges normal	3–30 µg./ml.	2.0–15 µg./ml.
Chloramphenicol <sup>9</sup>	12	<i>H. influenzae</i> meningitis	5–50 µg./ml.	5–25 µg./ml.
Streptomycin <sup>10, 11</sup>	9	Meninges normal	6.0–173 units/ml.	0.0–5 units/ml.
Streptomycin <sup>10, 11</sup>	2	<i>H. influenzae</i> meningitis and tuberculous meningitis	95.2–171 units/ml.	13.2–41.6 units/ml.
Dihydrostreptomycin <sup>4</sup>	4	Prophylaxis for prefrontal lobotomy; meninges normal	16–32 µg./ml.	0.25–1.00 µg./ml.
Tetracycline <sup>12, 13</sup>	>4	Meninges normal	2.5–10 µg./ml.	0–1.9 µg./ml.
Tetracycline <sup>14, 15</sup>	14	8 cases convalescent poliomyelitis; 6 cases severe soft-tissue infections; meninges not noted	0.85–40 µg./ml.	0.125–5.0 µg./ml.
Tetracycline <sup>16</sup>	19	<i>H. influenzae</i> purulent meningitis	0.38–24 µg./ml.	0.19–3.8 µg./ml.
Oxytetracycline <sup>4, 17</sup>	9	Meninges normal; includes 4 cases of prophylaxis for prefrontal lobotomy	4–80 µg./ml.	0.0–2.5 µg./ml.
Oxytetracycline <sup>14</sup>	5	Meninges not noted; convalescent poliomyelitis		0.63–1.250 µg./ml.
Oxytetracycline <sup>17</sup>	8	Meningitides: meningococcal (5), pneumococcal (1), <i>H. influenzae</i> (1), and undetermined (1)	0–80 µg./ml.	0.625–5.0 µg./ml.
Chlortetracycline <sup>4</sup>	4	Prophylaxis for prefrontal lobotomy; meninges normal	2–16 µg./ml.	0–0.0312 µg./ml.
Chlortetracycline <sup>14</sup>	3	Convalescent poliomyelitis; meninges not noted		0.1–0.2 µg./ml.
Novobiocin <sup>18</sup>	24	Meninges normal	3.56–90.7 µg./ml.	None
Penicillin <sup>4</sup>	1	Prophylaxis for prefrontal lobotomy; meninges normal	0.06–2.0 units/ml.	0–0.12 units/ml.
Penicillin <sup>19</sup>	162	Early syphilis; meninges not involved	10–25 million units intravenously per 24 hours	0–0.55 units/ml.
Penicillin <sup>20, 21</sup>	34	With and without meningitis	2.5–20 units/ml.	0.08–2.50 units/ml. during meningitis
Penicillin <sup>22</sup>		Purulent meningitis	500,000 units intravenously	2–8 units/ml.

There is great need therefore for antistaphylococcal agents against which staphylococci do not readily become resistant. Especially in cases of staphylococcal meningitis, such agents must be bactericidal in low concentrations. Resistance to ristocetin is apparently not rapidly acquired, cultures of *Staph. aureus* that are resistant to other antibiotics have been sensitive to ristocetin, and the antibiotic is bactericidal at about the same concentration at which it is bacteriostatic.<sup>1</sup> We can now add to these advantages the possibility that ristocetin may have therapeutic potency in the treatment of staphylococcal meningitis, justifying further trial.

#### SUMMARY

The use of ristocetin against infections of the central nervous system has not been previously reported, and chemical and animal studies indicated that normal meninges formed the same barrier as with most other antibiotics. However, in the case reported, other antibiotics had failed, and the patient was apparently moribund. Ristocetin and gamma globulin were administered, and a cure was effected. Studies of body fluids show that ristocetin passes the blood-brain barrier to give residual levels commensurate with concurrent serum levels.

#### ACKNOWLEDGMENTS

Drs. George C. Greene and James Jones, obstetricians, kindly referred the patient. The tracheostomy was performed by Dr. Joseph Ballard and the exploratory trephining by Dr. Ralph Angelucci. The ristocetin was supplied and the analyses of body fluids were performed by Abbott Laboratories.

#### BIBLIOGRAPHY

1. GRUNDY, W. E.; ALFORD, E. F.; RDZOK, E. J., AND SYLVESTER, J. C.: Ristocetin, the development of resistance and bactericidal activity. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 693.
2. PHILIP, J. E.; SCHENCK, J. R., AND HARGIE, M. P.: Ristocetins A and B, two new antibiotics: isolation and properties. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 699.
3. HWANG, K.; PRIMACK, N.; STEIN, R. J., AND RICHARDS, R. K.: Pharmacological and toxicological properties of ristocetins A and B. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 163.
4. WELLMAN, W. E.; DODGE, H. W., JR.; HEILMAN, F. R., AND PETERSEN, M. C.: Concentration of antibiotics in the brain, *J. Lab. & Clin. Med.* 43:275, 1954.
5. HEILMAN, F. R.; HERRELL, W. E.; WELLMAN, W. E., AND GERACI, J. E.: Some laboratory and clinical observations on a new antibiotic, erythromycin (Ilotycin), *Proc. Staff Meet., Mayo Clin.* 27:285, July 16, 1952.
6. ROMANSKY, M. J.; NASOU, J. P.; DAVIS, D. S., AND RITTS, R. E., JR.: Clinical spectrum of erythromycin as evaluated in two hundred sixty patients, *J.A.M.A.* 164:1197, July 13, 1957.
7. DEANE, G. E.; FURMAN, J. E.; BENTZ, A., AND WOODWARD, T. E.: Treatment of meningitis with chloromycetin palmitate, results of therapy in 23 pediatric cases, *Pediatrics* 11:368, April 1953.
8. KELLY, R. S.; HUNT, A. D., JR., AND TASHMAN, S. G.: Studies on the absorption and distribution of chloramphenicol, *Pediatrics* 8:362, 1951.
9. MCCRUMB, F. B., JR.; HALL, H. E.; MEREDITH, A.; DEANE, G. E.; MINOR, J. V., JR., AND WOODWARD, T. E.: Treatment of *Hemophilus influenzae* meningitis with chloramphenicol and other antibiotics, *J.A.M.A.* 145:469, Feb. 17, 1951.
10. ZINTEL, H. A.; FLIPPIN, H. F.; NICHOLAS, A. C.; WILEY, M. M., AND RHOADS, J. E.: Studies on streptomycin in man. I. Absorption, distribution, excretion and toxicity, *Am. J. M. Sc.* 210:421, Oct., 1945.
11. BUGGS, C. W.; PILLING, M. A.; BRONSTEIN, B., AND HIRSHFELD, J. W.: The absorption, distribution, and excretion of streptomycin in man, *J. Clin. Investigation* 25:94, Jan., 1946.
12. MAYNARD, A. DE L.; PRIGOT, A., AND ANDRIOLA, J. C.: Observations on the absorption, diffusion, and excretion of tetracycline hydrochloride. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 655.

13. SCHWARTZAR, S.; REEVES, R.; CLAPS, A., AND ANDERSON, A. F.: Tetracycline: studies on absorption, distribution, excretion, and clinical trial in children, *J. Pediat.* 45:285, Sept., 1954.
14. WOOD, W. S., AND KIPNIS, G. P.: The concentrations of tetracycline, chlortetracycline and oxytetracycline in the cerebrospinal fluid after intravenous administration. *In: Antibiotics Annual 1953-1954*, New York, Medical Encyclopedia, Inc., 1954, p. 98.
15. MAYNARD, A. DE L.; ANDRIOLA, J. C., AND PRIGOT, A.: Tetracycline hydrochloride: studies on absorption, diffusion, excretion, and clinical trial. *In: Antibiotics Annual 1953-1954*, New York, Medical Encyclopedia, Inc., 1954, p. 102.
16. HANSEN, H., AND KOCH, R.: Tetracycline serum and cerebrospinal fluid levels in acute purulent meningitis. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 319.
17. KOCH, R.: Oxytetracycline serum and cerebrospinal fluid levels in meningitis. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 908.
18. BAYNE, G. M.; STRICKLAND, S. C.; GYLFE, J. M., AND BOGER, W. P.: Novobiocin: a study of plasma and spinal fluid concentrations in man, *Antib. Med.* 2:166, March, 1956.
19. SCHWEMLEIN, G. X.; BARTON, R. L.; BAUER, T. J.; LOEWE, L.; BUNDESEN, H. N., AND CRAIG, R. M.: Penicillin in spinal fluid after intravenous administration, *J.A.M.A.* 130:340, Feb. 9, 1946.
20. DOWLING, H. F.; SWEET, L. K.; ROBINSON, J. A.; ZELLERS, W. W., AND HIRSH, H. L.: The treatment of pneumococcal meningitis with massive doses of systemic penicillin, *Am. J. M. Sc.* 217:149, Feb., 1949.
21. DOWLING, H. F.; SWEET, L. K.; HIRSH, H. L., AND LEPPER, M. H.: Specific therapy of bacterial infections of central nervous system, *J.A.M.A.* 139:755, March 19, 1949.
22. BOGER, W. P.: Abstract of discussion on: Specific therapy of bacterial infections of central nervous system, *J.A.M.A.* 139:755, March 19, 1949.
23. ROMANSKY, M. J.; NASOU, J. P.; DAVIS, D. S., AND RITTS, R. E., JR.: The treatment of one hundred and seventy-one patients with erythromycin, including one hundred and thirty-two with bacterial pneumonia. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, p. 48.
24. DAVIES, J. A. V.: The treatment of meningitis, *M. Clin. North America*, 29:1259, Sept., 1945.
25. PRICE, A. H., AND HODGES, J. H.: Treatment of meningitis with penicillin injected intravenously and intramuscularly, *New York J. Med.* 44:2012, pt. 2, Sept. 15, 1944.
26. HOLPER, J. C.; RICKHER, C. J., AND SYLVESTER, J. C.: Ristocetin: effect of gamma globulin on in vivo activity. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 577.
27. APPELBAUM, E., AND ABLER, C.: Advances in the diagnosis and treatment of acute pyogenic meningitis, part I, *New York J. Med.* 58:204, Jan. 15, 1958.
28. CARSON, M. J., AND KOCH, R.: Management of bacterial meningitis in children, *Pediat. Clin. North America* 3:377, May, 1956.
29. FINLAND, M., AND HAIGHT, T. H.: Antibiotic resistance of pathogenic staphylococci, *A.M.A. Arch. Int. Med.* 91:143, Feb. 1953.
30. FISHER, A. M.; TREVER, R. W.; CURTIN, J. A.; SCHULTZE, G., AND MILLER, D. F.: Staphylococcal pneumonia: a review of 21 cases in adults, *New England J. Med.* 258:919, May 8, 1958.

# Treatment of Pulmonary Abscesses and Chronic Pulmonary Suppurations with Oxytetracycline

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It may be surprising that a surgical clinic is reporting on the conservative therapy of inflammatory lung diseases. Resistant chronic pneumonias and pulmonary abscesses, withstanding internal therapy, as well as cases of suspected lung cancer were referred for treatment to our clinic.

From 1950 to 1957, a series of 320 patients with chronic pneumonia associated with abscess formation and pulmonary abscesses were studied. Of these patients, 141 were treated surgically. Ten more special patients of this group also underwent operations. Histological examination of 3 of the latter 10 patients revealed extended pneumonias with abscess formation associated with a carcinoma; the remaining 7 cases of nonspecific pneumonia were complicated by a specific tuberculosis pneumonia.

Of the 169 patients treated conservatively, 47 received oxytetracycline (Terramycin\*). The remaining patients were merely examined and transferred to a medical department for further treatment. In the chronic pneumonias associated with abscess formation, the diagnoses on admission, for the most part, were suspected lung cancer and lung cancer, respectively.

In all patients with pulmonary abscesses, the conservative therapy was carried out at our clinic, since the treatment performed elsewhere generally was inadequate. Only in those cases in which we had to deal with hemorrhages was an immediate operation indicated. In chronic pneumonias associated with abscess formation, the conservative treatment was started if the roentgenogram, the tomogram, the bronchoscopy, and, in special cases, the bronchography showed that the bronchi were not involved, and thus existence of a carcinoma was improbable. Robbin and Sniffen for the first time had referred to this very form of chronic pneumonia associated with abscess formation. Numerous papers hitherto appeared wherein surgical measures alone were recommended. Based on information in the Anglo-American literature and on a report by Niedner and Lange, Denk, former head of our clinic, suggested and introduced the therapy with oxytetracycline. In 1950, Jenny reported goods results achieved with penicillin in pulmonary abscesses due to staphylococci. The same author also described 15 cases of pulmonary complications following tonsillectomy that occurred within the years 1944 to 1954. Six of these cases were cured with the help of antibiotic therapy. The broad-spectrum antibiotic, oxytetracycline, was especially indicated for use in treatment, since, for the most part, the organisms were mixed flora with staphylococci, streptococci, and *Escherichia coli*.

Among the 47 patients treated only conservatively with oxytetracycline were 17 patients with solitary pulmonary abscesses and 28 with chronic pneumonia associated with abscess formation. These patients ranged in age from 23 to 83 years old; the majority, however, were in the fourth and fifth decades of life. The pulmonary abscesses were principally localized in the apex of the superior and inferior lobes, i.e., in 3 patients each in the right and left superior lobes, in 1 in the right middle lobe, and in 4 cases in the right and in 3 cases in the left apex of the inferior lobes. Only three times was a pulmonary abscess located in the basal segments of

\* The trade name of Chas. Pfizer & Co. for oxytetracycline is Terramycin.

the right inferior lobe. Of these, one abscess occurred subsequent to an accident and another postoperatively following cystectomy.

The dosage used was 1 Gm. oxytetracycline daily given by the oral route (in six hour intervals, one capsule of 250 mg.) simultaneously with B complex and vitamin K. Moreover, importance was attached to administration of cardiovascular stimulants and to an appropriate physical therapy to relieve expectoration (inhalation, change of position, and percussion of the affected parts of the chest).

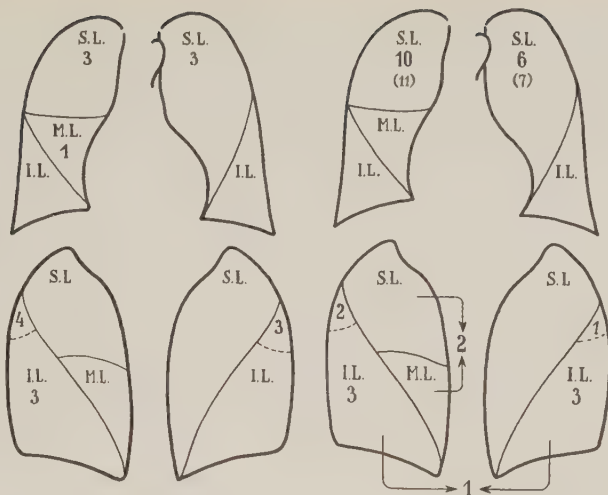
The patients usually were free of fever in two to three days; in a few exceptional cases they became afebrile after 8 to 12 days. The sputum quantity decreased within the first week. The drug was administered for at least 10 days, extending, in some cases, to one month. The abscesses were two to three inches in diameter; 9 abscesses were as large as apple-size.

In contrast with the good subjective improvement objectively manifest clinical and roentgenological lung findings improve very slowly. In the majority of cases, the abscess can no longer be demonstrated—even by roentgenogram—after 8 to 10 weeks. In general, it can be realized after 10 to 14 days whether the conservative treatment will bring about recovery. In 7 of our cases the final result was a clean cyst; however, it could not be stated with certainty whether a cyst of the lungs existed primarily and was infected secondarily. Case histories of these patients made no mention of pulmonary disease. To date, all of the 7 patients are healthy. The remaining 9 cases could be completely cured or healed with slight fibrous cicatricial tissue. One patient with a giant abscess died on the fifth day due to an arrhythmia hemorrhage.

Twenty-eight cases of chronic pneumonia associated with abscess formation (pneumonitis suppurativa and cholesterine pneumonia) were also treated conservatively with oxytetracycline. These processes were located as follows: in the right superior lobe, 10 cases; in the left, 6; in the apex of the inferior lobe, 2 right, 1 left; in the basal segments on the left, and on the right, 3 cases each. The superior and middle lobes were affected simultaneously in 2 cases, while in another 2 cases the process occurred in both inferior lobes at the same time. Also these changes, in general, are located in the superior lobe and apex of the inferior lobe, respectively. It is interesting to note that the right lobes are more frequently affected than the left, which was already outlined in Austria by Chiari and Denk. These patients also were treated with 1 Gm. oxytetracycline daily, B complex, vitamin K, and cardiovascular stimulants. In addition physical measures to relieve expectoration were employed simultaneously. It was striking that in these cases, too, defervescence generally occurred in two or three days, yet after one week at the latest, the sputum quantity decreased rapidly and subjective improvement progressed satisfactorily. The objective improvement of the lung and roentgenological findings were observed to occur later. Gastrointestinal tolerance of the drug was excellent; only one case of enterocolitis occurred, which, however, subsided rapidly after discontinuation of the drug. This complication, however, is in our experience extremely rare and does not influence us in the indication of antibiotics. In one case an urticarial exanthema was noted, but this did not necessitate discontinuation of therapy.

The final result of the conservative therapy in 2 cases was a shrunken lobe with scar tissue formation, yet without subjective and objective improvement. In 17 cases the process healed with fibrous cicatricial tissue. In another 9 cases, after healing was shown roentgenologically, no changes were proved. The primary roentgenogram in all cases revealed a chronic pneumonia associated with abscess formation and cavities up to 1.5 cm. (0.6 inches) in diameter.

FIG. 1. (Left) Localization of the pulmonary abscesses. (Right) Localization of the chronic pneumonia with abscess formation.



Two further cases of chronic pneumonia associated with abscess formation responded well to oxytetracycline treatment. After two months, however, a central carcinoma, which certainly had previously existed, became manifest in 2 patients, i.e., one in the right and one in the left inferior lobes, respectively. The secondary chronic pneumonia with abscess formation, which had been concealed behind a bronchostenosis, subsided under treatment with oxytetracycline and thus resulted in a temporary improvement.

The bacteriological cultures of the sputum in the abscesses and in the chronic pneumonias with abscess formation revealed a mixed flora with *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus viridans*, and *E. coli*. In one case, *Pseudomonas aeruginosa* was also a concurring cause.

Of course, oxytetracycline was given in surgical cases pre- and postoperatively, whereby much better results were achieved than in previous years. If, after a period of two weeks, no objective improvement is observed, an operation is indicated. In these cases the total duration of the disease was approximately 5 to 8 weeks. These indications are applicable to either group of the disease. We retain the conservative measures in those cases only in which, for other reasons, the surgical treatment is contraindicated.

#### CASE REPORT

The following is a report on a giant pulmonary abscess in which surgical treatment could be supported successfully with oxytetracycline.

A 57 year old turner and foreman, being in a very bad general condition, was admitted with a giant pulmonary abscess. The septic pulmonary infarction in question was fused in. The patient received orally 2 Gm. oxytetracycline daily for two weeks. Because of the usefulness of internal therapy, we decided to perform a pneumonotomy for opening the abscess. After 16 days, the abscess was only half as large as before, and after a further seven weeks the patient was discharged with a bronchial fistula. After a holiday he was fit for work again; in about one year the bronchial fistula healed. As indicated, the patient was given a total of 70 Gm. of the drug. No side reactions occurred. The bacterial culture of the sputum revealed *Staph. albus*, *E. coli*, and *Bacterium xerosis*; in the abscess, *Proteus vulgaris*, *Ps. aeruginosa*, and *Staph. albus* were found. At this time, five years after treatment, the patient is healthy, has gained a total of about 50 lb., and is fully fit for work.

#### CONCLUSIONS

On the basis of the excellent results obtained in 47 cases of chronic pulmonary

inflammations, it may be stated in conclusion that treatment with oxytetracycline brings about rapid improvement of subjective symptoms. Pathological changes, which were manifest clinically and roentgenologically, disappeared only slowly. The success must be judged very cautiously. Even after improvement of all inflammatory symptoms, the possibility of a malignant process must be considered and the patient carefully followed up.

A conservative therapy alone, not bringing about a marked regression of the relevant changes, must not be employed longer than three weeks.

In cases in which oxytetracycline is indicated, this drug produces excellent results. While, in the pre-antibiotic era, only those lung abscesses smaller than plum-size could be treated conservatively, it is now possible to treat successfully abscesses with a diameter of 2 inches and larger with oxytetracycline.

#### BIBLIOGRAPHY

1. CHIARI, H.: *Langenbecks Arch. u. Dtsch. Z. Chir.* 268:125-149, 1951.
2. DENK, W.: Personal information.
3. DENK, W.: *Langenbecks Arch. u. Dtsch. Z. Chir.* 268:150-164, 1951.
4. JENNY, R. H.: *Wien. klin. Wchnschr.* 61:5, 1949.
5. JENNY, R. H.: *Wien. klin. Wchnschr.* 66:24, 1954.
6. NIEDNER, F. F., AND LANGE, H. P.: *Dtsch. med. Wschr.* 77:8, 1952.
7. ROBBIN, L. L., AND SNIFFEN, R. C.: *Radiology* 53:187, 1949.

# Ristocetin in the Treatment of Seven Selected Difficult Cases

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Ristocetin has produced excellent results in eradicating, mitigating, or preventing infection in 7 selected difficult cases. Five cases involved strains of *Staphylococcus aureus*, which did not respond to chemotherapy with other antibiotics. Three of the 5 cases were severe chronic osteomyelitis and 2 were severe skin infections. One was multiple myeloma complicated by peritonitis which followed perforation of the colon and one was a case of chronic ulcerative colitis. Bacteria were not isolated from these 2 cases. All of these cases are presented as illustrations of the use of ristocetin, a new antibiotic effective against gram-positive organisms, especially resistant strains of staphylococci.

## CASE REPORTS

*Case 1.* A 24 year old Negro man was admitted with osteomyelitis of the right tibia. Two years previously he had suffered a compound comminuted fracture of the right tibia and fibula. This had been treated by closed reduction and immobilization with a plaster cast and had been followed by open reduction with fixation with an intramedullary nail and a bone graft. After removal of the nail another cast had been applied.

At the present admission a purulent sinus existed at the site of fracture from which *Staph. aureus* and *Pseudomonas* species were isolated. Streptokinase, 20,000 units, was given buccally every 12 hours for four days. Then, erythromycin, 250 mg., was given by mouth every six hours for 18 days. During this time, a curettage of the right tibia was done. Trypsin, 5 mg., was administered buccally every 12 hours for four days. Provided with a moulded cuff brace, the patient was discharged after a total stay of eight weeks.

One month later the patient reinjured his leg, received initial treatment elsewhere, and was readmitted with purulent drainage at the original site. Penicillin, 300,000 units, and streptomycin, 0.5 Gm., were given intramuscularly twice daily for 17 days. A small abscess at the site of fracture was incised and drained and a *Staph. aureus* was identified. Chloramphenicol, 250 mg., was given by mouth every four hours for 25 days and this was followed by erythromycin, 250 mg., by mouth every four hours for 23 days. A sequestrectomy was then performed and was followed by concurrent therapy with chloramphenicol, 250 mg., by mouth every four hours for one month, and erythromycin, 250 mg., by mouth every four hours for six weeks.

Despite this treatment drainage persisted, so ristocetin was given at a rate of 1.0 Gm. intravenously every 12 hours for seven days. Drainage stopped, the wound healed, and a walking-type plaster cast was applied. The patient was discharged 10 days after therapy with ristocetin ended. The cast was removed one month following release from the hospital. Five months later the wound was still healed, and roentgenographic evidence of osteomyelitis was not present.

*Comment.* This patient had evident chronic osteomyelitis uninterruptedly for about two and one-half years. During this period, he received excellent treatment along with the administration of antibacterial drugs. Healing, however, was achieved only after treatment with ristocetin. This wound has remained healed for five months and the result to date can be considered as excellent.

*Case 2.* A 36 year old Negro man was admitted with an osteomyelitis and purulent drainage of the left supracondylar region. Eighteen months previously he had incurred a compound comminuted fracture of the left femur and a fracture of the right hip. The right hip had been treated by open reduction and internal fixation. The left femur was débrided, reduced, and im-

mobilized by a plaster cast. The wound on the left did not heal completely and a sequestrectomy was required. Subsequently, a bone graft was applied.

On the present admission, a sinus with purulent exudate was present. *Staph. aureus* was isolated from the pus. The left leg was 7.5 cm. shorter than the right, and the roentgenograms showed an osteomyelitis and non-union at the site of the fracture. There was also a completely ankylosed left knee joint.

Chloramphenicol, 250 mg., was given by mouth every four hours for two weeks. The Smith-Petersen nail and the McLaughlin plate were removed from the right femur. Following this penicillin, 300,000 units, and streptomycin, 0.5 Gm., were given intramuscularly every 12 hours for three weeks, and erythromycin, 250 mg., by mouth every four hours for one month subsequently. The exudate from the left femur persisted, so treatment with ristocetin, 1.0 Gm. twice daily, was initiated. At the end of two weeks of therapy with ristocetin the purulent discharge decreased sufficiently to allow a left supracondylar amputation. The wound healed well as ristocetin was continued for one week postoperatively. Three months later, after rehabilitation and the fitting of a prosthesis, the patient was discharged.

*Comment.* The left leg of this patient was useless and an osteomyelitis was present. Amputation and the use of a prosthesis were indicated as the best means of rehabilitation. The infection was effectively controlled by ristocetin, the amputation was successful, and the wound healed without incident.

*Case 3.* A 22 year old white man was admitted with an osteomyelitis of the right tibia. The right tibia and fibula had been fractured two years previously. Treatment prior to admission had included a closed reduction and application of a plaster cast, which had been followed by an open reduction with intramedullary fixation. The patient then fell and damaged the nail, which required its removal and the application of a bone graft. The surgical wound had healed with the exception that there were multiple, small, and purulent sinuses present on admission.

On culture, *Staph. aureus* was identified. Chloramphenicol, 250 mg., was given by mouth every four hours for 26 days. Because a response was not obtained, this was followed by ristocetin, 1.0 Gm., every 12 hours for 28 days. By the fourth day of therapy with ristocetin, drainage was greatly decreased. On the eighteenth day the wound was saucerized. Healing progressed slowly for five weeks. Chloramphenicol, 250 mg. was given by mouth every four hours for five weeks, erythromycin, 250 mg., by mouth every four hours for two weeks, and oxytetracycline, 250 mg., by mouth every four hours for three weeks. Three months after saucerization, the patient was discharged. Four months after his release the wound had become smaller and cleaner, and a roentgenogram revealed quiescence of the osteomyelitis.

*Comment.* The end result in this patient is good, and only skin closure remains to be effected. Although the wound is not completely healed, it is smaller and aseptic. The purulent drainage has stopped and the osteomyelitis is quiescent as shown by roentgenograms. The same result might have been achieved without ristocetin but the course of the other drugs was so prolonged that short-term ristocetin therapy was preferred.

*Case 4.* A 58 year old white man had a small ulcer in the left floor of the mouth after having received roentgen treatment for a squamous-cell carcinoma. The lymph nodes in the left and the right sides of the cervical region were enlarged. The ulcer was excised, part of the exposed left side of the mandible was removed, and the floor of the mouth was closed. Tetracycline, 250 mg., was given by mouth every six hours for six days, withdrawn for 11 days, and administered again for 10 days.

Following this, a radical excision of the left cervical region and a minimal excision of the left side of the mandible were performed. The area was drained by air-vent suction through two urethral catheters. Penicillin, 300,000 units, and streptomycin, 0.5 Gm., were given intramuscularly every 12 hours for one month. During this time, the wound became infected and a *Staph. aureus* was isolated from the pus. The infection, however, regressed satisfactorily under continued treatment with the same drugs.

Later, a large infected area formed on the right anterior chest and a *Staph. aureus* was again isolated. Ristocetin, 875 mg. twice daily, was given intravenously for two weeks, and the infection healed without event.

Two weeks later streptokinase, 20,000 units buccally twice daily, was given for five days. Ristocetin in the same dosage was started at the same time and continued for two weeks. A radical excision of the right cervical region was done. Other chemotherapy included penicillin, 300,000 units, and streptomycin, 0.5 Gm., which was given intramuscularly every 12 hours for various intervals up to two weeks. The wound healed without event.

Six weeks later a small area of the left side of the mandible was excised because of osteomyelitis. Streptokinase was given for three weeks. This wound did not heal completely but the patient was discharged five weeks after the excision.

*Comment.* The use of the other antibiotics had created a favorable situation for the growth of *Staph. aureus* in this patient, and a persistent area of infection in the mouth aided the spread of foci. Thus, it was not surprising that there were staphylococcal infections of the wound in the left neck and in the left chest wall. The infection of the chest wall responded very well to ristocetin alone and the right side of the neck was resected under cover of ristocetin without incident. This was an excellent result from the use of this antibiotic.

*Case 5.* A 38 year old white man was admitted, from another hospital, in a semi-comatose and disoriented state following a head injury in an automobile accident. Infections were present involving the right lumbar region and the outer anterior aspect of the right leg. Therapy was instituted with penicillin, 300,000 units, and streptomycin, 0.5 Gm., intramuscularly every 12 hours for six days. The lumbar abscess was incised and drained and a *Staph. aureus* was isolated from the pus. Five days later the other abscess was incised and drained and the same bacteria identified. Streptokinase, 5000 units, was given intramuscularly every 12 hours for intermittent periods during the next three months. Four days after the first incision and drainage, 1.0 Gm of ristocetin was given intravenously, but the appearance of a papular rash on both legs caused its withdrawal. After the second incision and drainage, tetracycline, 250 mg., was given by mouth for one week. The wounds were indolent and multiple areas of infection formed on the face, left arm, and right lumbar region. Eighteen days after the administration of the tetracycline was stopped, ristocetin was started intravenously at a dose of 1.0 Gm. every 12 hours. This was continued for 12 days by which time all of the infections healed. The dermatitis did not recur.

The patient was kept in the hospital for rehabilitation, however, during which time a left epididymo-orchitis was noted. Tetracycline, 250 mg., was given by mouth for 10 days, and the infection cleared readily. Release from the hospital followed after one month.

*Comment.* This patient was unconscious because of a head injury and had received antibiotics prophylactically. Often such therapy causes the overgrowth of resistant staphylococcal strains. In this patient multiple small infections, resistant to treatment with ordinary antibiotics, became established. A skin reaction was first thought to be caused by ristocetin but was probably caused by the penicillin that had been given elsewhere, since the second course with ristocetin was given without incident. The patient had an excellent response to treatment with ristocetin.

*Case 6.* A 37 year old white man was hospitalized with a pathologic fracture of the right hip. He had had a previous diagnosis of multiple myeloma and had undergone a laminectomy and partial excision of the tumor followed by roentgen treatment.

Well-leg traction was applied for the fracture. On the fourth day in the hospital, the patient had an elevation of the temperature. A persistent leukocytosis of about 17,000 white blood cells per cu. mm. with a shift to the left was present. A focus of infection was not uncovered. Empirically, streptomycin, 0.5 Gm. intramuscularly every 12 hours, penicillin, 300,000 units intramuscularly every 12 hours, and chloramphenicol, 250 mg., by mouth every six hours, were given for varying periods of time from the fourth through the twenty-ninth day.

On the thirty-fourth day, the patient vomited a bloody material. On the following day, he complained of severe abdominal pain, his white blood cell count was 15,500 cells/cu. mm. with a shift to the left, and acute peritonitis was diagnosed. At the time of operation, feces were noted entering the peritoneal cavity through a perforation of the ascending colon. The entire colon was inflamed and extremely fragile. The diagnosis was a pseudomembranous enterocolitis. The perforation was sutured and a cecostomy made. Drainage was instituted with air-vent suction by catheters at multiple sites.

Tetracycline, 500 mg. intravenously, was administered on the day of surgery. Streptomycin, 0.5 Gm., intramuscularly every 12 hours, was given from the day of surgery through the nineteenth postoperative day. On the second postoperative day, with the white blood cell count at 16,700 cells/cu. mm., ristocetin was instituted at a dose of 750 mg. intravenously every 12 hours. This was continued through the fourteenth postoperative day when the white blood cell count had dropped to 9150 cells/cu. mm. During this course of therapy the patient recovered slowly from his serious infection.

On the thirty-fourth postoperative day, a rectal hemorrhage signaled the return of infection and the blood pressure fell to shock levels. Whole blood was transfused, and ristocetin was reinstituted for a period of five days. The hemorrhage stopped but it recurred five days later. Ristocetin was again given for three days and again the bleeding came under control.

On the following day, ristocetin, 100 mg., combined in a tablet with neomycin, 250 mg., was given orally every 12 hours for five days, followed by two tablets every 12 hours for 15 days. Since the enterocolitis was limited to the inner lining of the colon and these drugs are not absorbed through the intestinal tract, it was hoped that the infection could be controlled by this use of local therapy in the same manner as preoperative sterilization is done.<sup>6</sup> The therapy was successful and the stools became quickly negative for occult blood.

Six weeks later, however, pneumonia occurred. Tetracycline, 250 mg., was given by mouth every six hours at various intervals over the next six weeks. Terminally the patient received chloramphenicol, 250 mg., by mouth for two days. Death finally resulted from the pneumonia, which complicated the underlying multiple myeloma.

*Comment.* This patient incurred an enterocolitis presumably from the administration of antibacterial drugs. The perforation of the colon produced a violent peritonitis and a prolonged serious illness. Intravenous ristocetin definitely contributed to his initial recovery.

Therapy was changed to a combination of ristocetin and neomycin in oral form. The final eradication of colonic disease was effected by this therapy. The eventual death of the patient from multiple myeloma and its complications could not be prevented.

*Case 7.* A 26 year old white man was admitted complaining of abdominal cramps and 10 to 12 liquid stools daily which occasionally contained blood. Four years previously the diagnosis of chronic ulcerative colitis had been made.

Proctoscopic examination revealed a rectal polyp and an inflamed mucosa with multiple minute ulcerations. Roentgenograms following a barium enema showed a chronic ulcerative colitis. An air-contrast examination with barium enema did not reveal other polyps.

Treatment was started by the oral administration of a combination of ristocetin, 100 mg., and neomycin, 250 mg. Two tablets of this combination were given twice daily for 19 days. The abdominal cramps subsided by the third day of therapy, stools decreased to two per day, although they remained liquid, and gross blood disappeared from the feces. The rectal polyp was removed on the thirteenth day of chemotherapy, and the patient was discharged one month later in remission. Three months after discharge the patient had no complaints, and a proctoscopic examination was normal.

*Comment.* The excellent remission in this patient with chronic ulcerative colitis may have resulted from one of three factors in the treatment: the hospital environment, the administration of ristocetin, or the administration of neomycin. It would be difficult to assess the actual value of each factor. The sterilization afforded by this combination is quite complete as has been shown elsewhere,<sup>6</sup> albeit temporary. The combination of ristocetin and neomycin should be investigated further in the treatment of this disease.

#### DISCUSSION

Although the antibiotics have greatly improved the prognosis of chronic osteomyelitis, the treatment of this purulent condition is nonetheless difficult. Currently the seriousness of this infection and the difficulty of treatment, due in many cases

to unknown factors in host resistance, are complicated by the emergence of staphylococcal strains which are resistant to the more commonly used antibiotics. Thus, it is propitious that there should now be available a new antibiotic, ristocetin, which is especially effective against the *Staphylococcus* and to which a resistant strain is not known.

The 3 cases of chronic osteomyelitis described herein demonstrate the effectiveness of ristocetin against a staphylococcal organism, which resisted antibiotics administered previously. It controlled the infections preoperatively and provided a good cover for sequestrectomy. Postoperatively it kept the wounds clean and promoted healing. A dosage of 2 Gm. daily in two intravenous doses of 1.0 Gm. each was employed in all cases, and reduction of the dosage or withdrawal of the drug was not necessary because of side effects.

The 2 cases of multiple skin infections demonstrate the occurrence of infections in spite, or because, of the use of antibiotics. In one case antibiotics were administered prophylactically to a semi-comatose patient. This allowed the proliferation of staphylococcal organisms and the formation of foci of infection. Penicillin, streptomycin, and tetracycline were not successful in controlling the infections, and new foci became established even during treatment with these drugs. Healing of the infections occurred during the administration of ristocetin intravenously along with the enzymatic débriding agent, streptokinase, intramuscularly. In one case antibiotics had been administered to control the infection in a patient with a carcinoma, but the infection persisted, and even spread to various other areas of the skin. The use of penicillin and streptomycin brought satisfactory regression of the cervical infection. At the same time a large infection formed on the right anterior chest, however, and this required the administration of ristocetin. Then this infection healed promptly. Later streptokinase was given for its effect on inflammation and edema and ristocetin was administered to provide cover for radical excision in the cervical region. Penicillin, streptomycin, ristocetin, and streptokinase promoted healing without event.

A unique principle which 2 of these cases demonstrate is that local antibiotic therapy with ristocetin-neomycin tablets may be of help in intestinal disease. All therapeutic benefit observed in these cases had to be due to local antiseptics, since the drugs do not attain therapeutic blood levels by the oral route. The question as to which antibiotic was responsible for the effect, or whether, indeed either one alone would do as well as the combination, remains to be further delineated. In the case of chronic ulcerative colitis even more controls are required for a final verdict.

The resolution of inflammation and edema was enhanced by the use of streptokinase and trypsin. These proteolytic enzymes were given buccally.

#### SUMMARY

Several consecutive cases in which treatment with ristocetin was employed were presented and were discussed. Three cases of chronic osteomyelitis responded promptly. Two cases represented situations in which infections in the skin were fostered by therapy with other antibiotics and it remained for ristocetin, which was given intravenously, to eradicate the *Staphylococcus aureus*. There were 2 cases of disease of the colon in which tablets of ristocetin and neomycin, which were given orally, were used with benefit and in one of these the oral preparation helped maintain control of an infection that was first treated with ristocetin, given intravenously. These uses of ristocetin suggested that this agent has a wide application.

1. GRUNDY, W. E.; SINCLAIR, A. C.; THERIAULT, R. J.; GOLDSTEIN, A. W.; RICKHER, C. J.; WARREN, H. B., JR.; OLIVER, T. J., AND SYLVESTER, J. C.: Ristocetin, microbiologic properties. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 687.
2. GRUNDY, W. E.; ALFORD, E. F.; RDZOK, E. J., AND SYLVESTER, J. C.: Ristocetin, the development of resistance and bacterial activity. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 693.
3. ROMANSKY, M. J.; LIMSON, B. M., AND HAWKINS, J. E.: Ristocetin: a new antibiotic—laboratory and clinical studies. Preliminary report. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 706.
4. SCHNEIERSON, S. S.; AMSTERDAM, D., AND BRYER, M. S.: Bacterial sensitivity to ristocetin, *Antib. & Chemo.* 8:204, 1958.
5. CALVY, G. L., AND SCHUMACHER, L. R.: Ristocetin and the staphylococcus. Observations of effects and side-effects in seventeen cases of staphylococcic pneumonia, *J.A.M.A.* 167:1584, 1958.
6. SHIDLOVSKY, B. A.; PRIGOT, A., AND TURELL, R.: Influence of ristocetin on the aerobic intestinal flora of man. A preliminary report. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 199.

# Clinical Observations on Ristocetin

## A Preliminary Report on Its Efficacy and Toxicity in 20 Unselected Severe Respiratory Infections

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Ristocetin,\* a relatively new antibiotic produced from the actinomycete *Nocardia lurida*, was used in the treatment of 20 patients with upper respiratory infections. It is the purpose of this communication to report our experience as to the therapeutic efficacy of this antibiotic, as well as the side effects and toxicity encountered among our patients. Investigators elsewhere have reported fully on the pharmacology and toxicity of the drug.<sup>1,2</sup> Others have documented its effectiveness as an antistaphylococcal agent,<sup>3</sup> for presurgical intestinal antisepsis,<sup>4,5</sup> and in antibiotic-resistant infections.<sup>6</sup>

No attempt was made to screen the patients for this study with respect to bacteriological diagnosis or previous treatment. They were chosen at random, the only objective criteria for selection being severe toxicity, fever, and predominating symptoms of upper respiratory illness.

### MATERIALS AND METHODS

The 20 patients who were subjects in this study ranged in age from 13 to 81 years old. There were 13 men and 7 women. Radiological diagnosis was definitive for bronchopneumonia or lobar pneumonia in 19 cases and presumptive in 1.

Additional diagnoses were epilepsy in 2 patients and 1 case each of diabetic acidosis, bronchial asthma, peripheral neuritis, pleural effusion, and hypertensive cardiovascular disease with heart failure. Two patients had associated severe kidney insufficiency, 2 eventually were found to have tuberculosis (positive sputum cultures), and 1 had extensive pulmonary malignancy. One patient was a narcotics addict, and chronic alcoholism was a factor in 4 patients, with delirium tremens present or imminent in 2. Another patient presented, in addition to bronchopneumonia, systemic pathology that included possible tuberculosis, hypochromic anemia with anicytosis, and hepatomegaly.

Blood cultures were negative in 19 patients, while *Escherichia coli* was revealed in 1. Sputum cultures in 16 patients were not specific. In 2 cases alpha-hemolytic streptococci were isolated, and in 1 of these, *Staphylococcus albus* and pneumococcus were also identified.

Roentgenograms, urinalyses, and hematological studies were routine procedure throughout this investigation. Liver and kidney function tests and electrocardiographic examinations were also performed as indicated.

Ristocetin was available for intravenous use only. The powdered antibiotic, 500 mg. per vial, was dissolved in 500 ml. of Ringer's lactate and administered by the drip technique within the time limit of 30 to 45 minutes. In the majority of cases this was accomplished in the more desirable period of 30 minutes.

Average daily dosages ranged from 500 mg. every eight hours to 500 mg. every

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This investigation was supported, in part, by a grant from the Abbott Laboratories, North Chicago, Ill.

\* The trade name of Abbott Laboratories for ristocetin is Spontin.

TABLE I

## Twenty Patients with Severe Upper Respiratory Infections Treated Intravenously with Ristocetin

Patient no.	Age, yr.	Sex	Diagnosis	Associated pathology	Cultures		Medication		Side reactions	Clinical course
					Sputum	Blood	Dosage, mg. every 8 hr.	Duration, days		
1	45	M	Lobar pneumonia	Tuberculosis, epilepsy, renal insufficiency (patient transferred)	Acid-fast bacilli, tuberculous	<i>E. coli</i>	500	5	Maculopapular rash	Initial temperature response, again elevated in 72 hours; rash occurred, drug discontinued on fifth day; improvement in pneumonic process; fair response
2	35	F	Broncho-pneumonia	None	Neg.	Neg.	500	5	Temporary thrombocytopenia; normal in one week	Afebrile in 24 hours; excellent response
3	50	M	Lobar pneumonia	Chronic alcoholism, impending delirium	Neg.	Neg.	500 750	2 2	None	Temperature 103 to 104 F.; no response; patient died two days after course of medication
4	40	F	Lobar pneumonia	Diabetic acidosis	Neg.	Neg.	500	3	Temporary leukopenia; normal in three days	Afebrile in 24 hours; general response good
5	38	M	Broncho-pneumonia	Bronchial asthma	Neg.	Neg.	500	7	None	Gradual defervescence second to fourth day; normal on fifth day; excellent response
6	35	F	Upper respiratory infection; possible pneumonia	Tuberculosis (patient transferred)	Acid-fast bacilli, tuberculous	Neg.	500	3	None	Upper respiratory infection responded to medication, although temperature persisted
7	56	M	Lobar pneumonia	Probable pulmonary neoplasm, possible tuberculosis	Neg.	Neg.	500	3	None	Temporary temperature decline, again elevated; roentgenogram revealed probable neoplastic involvement; improvement in pneumonic process
8	28	M	Lobar pneumonia	None	Neg.	Neg.	500	3	None	Afebrile in 24 hours; excellent response
9	42	F	Lobar pneumonia	Slight pleural effusion	Neg.	Neg.	500	4	None	Afebrile in 24 hours; excellent response
10	81	F	Broncho-pneumonia	None	Neg.	Neg.	500 500°	3 4	None	Temperature fell initially, rose after 24 hours; excellent response after increased dosage; oldest patient in series

Table I Continued on Page 449

TABLE 1 (Continued)  
Twenty Patients with Severe Upper Respiratory Infections Treated Intravenously with Ristocetin

Patient no.	Age, yr.	Sex	Diagnosis	Associated pathology	Cultures		Medication		Side reactions	Clinical course
					Sputum	Blood	Dosage, mg. every 8 hr.	Duration, days		
11	13	M	Lobar pneumonia	None	Neg.	Neg.	250	7	None	Afebrile on fourth day; excellent response; youngest patient in series
12	45	M	Broncho-pneumonia	Possible tuberculosis	Neg.	Neg.	500	6	None	Temperature fell 105 to 102 F., but remained at that level; roentgenogram revealed possible tuberculosis
13	21	M	Broncho-pneumonia	Narcotic addiction, epilepsy	Neg.	Neg.	500	5	None	Afebrile in 24 hours; excellent response
14	48	M	Lobar pneumonia	Chronic alcoholism, peripheral neuritis	Neg.	Neg.	500 1 Gm.	3 4	None	Recalcitrant to prior antibiotic therapy; fever persisted even with high ristocetin dosage; poor response
15	60	M	Broncho-pneumonia	Cardiovascular disease, renal insufficiency	Neg.	Neg.	500 <sup>o</sup> 750 <sup>o</sup>	2 2	None	Recalcitrant to prior antibiotic therapy; fever persisted despite increased ristocetin; died of heart failure two days after course of medication
16	33	M	Broncho-pneumonia	Chronic alcoholism, delirium tremens	Pneumococcus, <i>Staph. albus</i> , Alpha-hemolytic <i>Streptococcus</i>	Neg.	500 <sup>o</sup>	4	Temporary leukopenia; normal in one week.	Prompt defervescence; excellent response
17	45	M	Broncho-pneumonia	Possible tuberculosis, anemia, hepatomegaly	Alpha-hemolytic <i>Streptococcus</i>	Neg.	500 <sup>o</sup> 759	3 †	Chills and vomiting on increased dosage	No response after three months treatment with various antibiotics of which ristocetin was last; discharged with temperature at lowest level 100.6 F.
18	65	M	Lobar pneumonia	Chronic alcoholism	Neg.	Neg.	500	5	None	Prompt and excellent response
19	35	F	Lobar pneumonia	None	Neg.	Neg.	500	4	None	Afebrile in 24 hours; excellent response
20	26	F	Lobar pneumonia	None	Neg.	Neg.	250 500	1	None	Afebrile in 48 hours; excellent response

<sup>o</sup> Dosage every six hours.  
† Single dose.

six hours. Two patients received initial doses of 250 mg. Whenever it was evident that the patient was not responding at the initial level, the dosage of the antibiotic was increased.

We wish to stress the point made by other investigators that, since ristocetin is excreted mainly by the kidneys, it should be used with great caution in patients with renal insufficiency. To minimize toxic side effects, careful attention to dosage and technique of administration is necessary. Since leukopenia and thrombocytopenia may develop as complications of ristocetin therapy, it is imperative that complete hematological determinations be made at frequent intervals.

## RESULTS

Pertinent data on our patients and the results of treatment are presented in table I.

*Therapeutic Response.* Our results with ristocetin were excellent among 13 of our 20 patients. They responded promptly to therapy, became afebrile, less toxic, and demonstrated radiological resolution of pneumonic processes. The average duration of medication was 4.8 days.

Analysis of the remaining 7 patients revealed that grave antecedent pathology complicated the clinical course in every instance. However, in 2 patients (1 and 6), who were found to have tuberculosis, ristocetin produced improvement in the pneumonic involvement, and they were subsequently transferred to the tuberculosis service. One patient with associated pulmonary cancer (patient 7) showed regression of the pneumonic infiltration while under treatment, but his general status remained unchanged. Another (patient 3), a chronic alcoholic with impending delirium tremens, failed to respond to medication and died two days after treatment was discontinued. Still another (patient 15), admitted with severe heart failure in addition to bronchopneumonia, eventually died of cardiovascular disease. Possible tuberculosis, severe anemia, and hepatomegaly complicated another (patient 17), this patient failing to respond to any antibiotic therapy administered over a period of three months. The remaining one (patient 14), with a primary diagnosis of lobar pneumonia and antecedent alcoholism and peripheral neuritis, had also been treated unsuccessfully with various antibiotics and derived no benefit when transferred to ristocetin, even at the high dosage level of 3 Gm. daily.

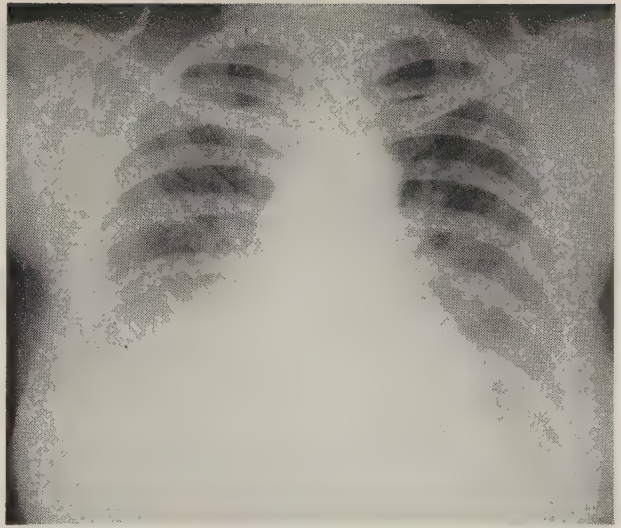
The following cases represent the typical response to ristocetin in the majority of patients under this investigation.

## CASE REPORTS

*Patient 19.* A. P., a 35 year old woman, was hospitalized on August 25, 1958, with a temperature elevation of 106 F., a productive cough, and chills. Roentgenogram on admission revealed right lower lobe pneumonia (fig. 1). Treatment with ristocetin was instituted at a dosage of 500 mg. every eight hours. The patient was afebrile 24 hours later. Medication was continued for three additional days with regression of all symptoms of infection. A roentgenogram taken on September 3, 1958, showed complete resolution of right lobar pneumonia (fig. 2).

*Patient 20.* E. D., a 26 year old woman, was admitted on August 23, 1958, with a three day history of fever, chills, cough, and chest pain. Her temperature was 104.6 F. Left lower lobe pneumonia was established by the initial roentgenogram (fig. 3). Ristocetin was administered at 250 mg. every eight hours for the first day. On the second day the dosage was in-

FIG. 1. This is roentgenogram made on August 25, 1958, showing lobar pneumonia, right lower lobe, at the time of admission. (Case A. P.)



creased to 500 mg. every eight hours. Her temperature was reduced on the following day from 104.6 to 101.6 F., and defervescence was complete three days later. Re-examination of the chest by roentgenogram on September 3, 1958, showed complete resolution of left lobar pneumonia (fig. 4).

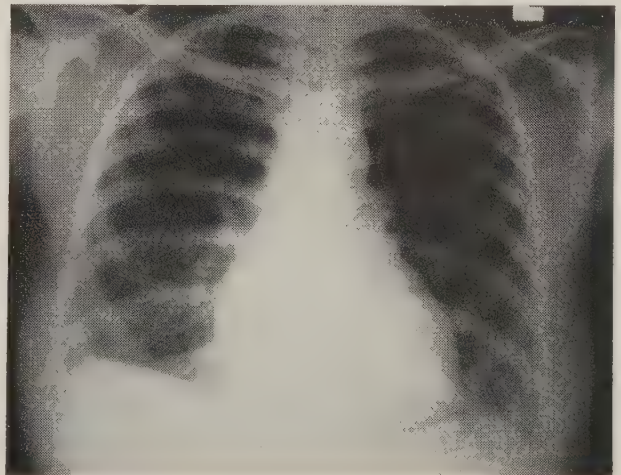
#### SIDE EFFECTS AND TOXICITY

There was no instance of local irritation or phlebitis from the intravenous administration of ristocetin. Adverse systemic reactions to ristocetin encountered in this series of cases were not serious and were transitory in character.

One patient had a diffused erythematous maculopapular rash and another complained of chills and vomiting. In both cases, the symptoms were temporary and disappeared promptly with cessation of medication.

Throughout this investigation complete blood counts were performed at frequent intervals. Among the 20 patients were 2 who developed thrombocytopenia, with readings of 146,000 and 132,000 cells/cu. mm., respectively, which returned to normal values of 300,000 and 270,000 cells/cu. mm. one week later. In another patient, a leukopenia of 3600 cells/cu. mm. reverted to normal level of 6700 cells/cu. mm. within six days.

FIG. 2. This second roentgenogram, made on September 3, 1958, after treatment with ristocetin, shows complete resolution of the lobar pneumonia. (Case A. P.)



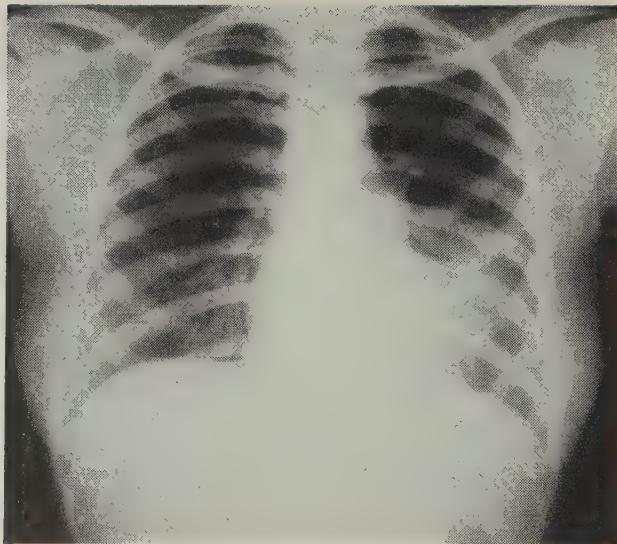


FIG. 3. Lobar pneumonia, left lower lobe, is seen in this roentgenogram made on August 23, 1958. (Case E. D.)

There were no other untoward reactions. Laboratory studies revealed no evidence of liver or kidney damage. Routine roentgenograms and electrocardiograms showed no undesirable changes attributable to the antibiotic.

The following reports present in some detail 2 cases in which temporary abnormalities of the cellular elements of the blood were observed.

#### CASE REPORTS

*Patient 2.* B. B., a 42 year old woman, was admitted with a three day history of chills and fever and pain of the right lower chest region. Physical examination revealed the patient to be toxic, slightly confused, dehydrated, and with a temperature of 103.4 F. The rest of the examination was negative, except for evidence of fine crepitant râles of the right lower lobe. Roentgenogram revealed right lower lobe pneumonia. Laboratory findings were noncontributory. The patient was started immediately on ristocetin in Ringer's lactate at a dosage of 500 mg. every eight hours. General improvement was noted after 24 hours of treatment. The patient was alert and lucid. Temperature dropped to 100 F. On the third day of medication, the platelet count showed a decline to 146,000 cells/cu. mm., although the rest of the hematological studies were normal. The drug was immediately discontinued. Blood studies were normal seven days later, the platelet count being 300,000 cells/cu. mm. The patient made an uneventful recovery.

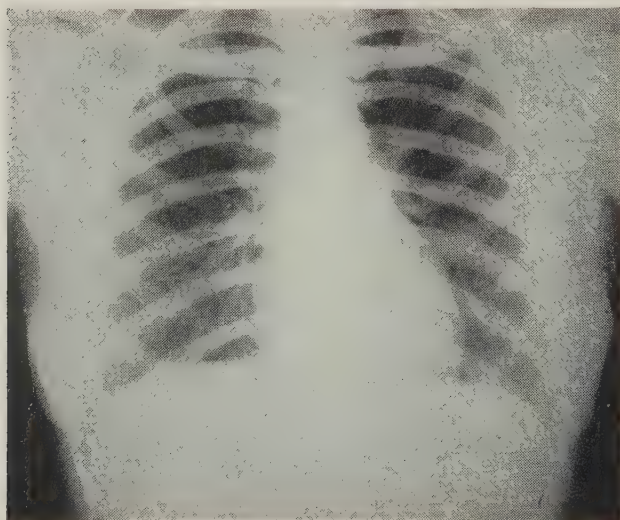


FIG. 4. Complete resolution of the lobar pneumonia, left lower lobe, is demonstrated on this roentgenogram made on September 3, 1958, after ristocetin treatment. (Case E. D.)

*Patient 16.* T. M., a 33 year old man, was hospitalized with a history of chronic alcoholism and a cough and fever of four days' duration. The patient was disoriented and extremely agitated, complaining of seeing "bugs." Physical examination revealed a severely toxic patient, with a typical picture of delirium tremens. The remainder of the findings of the initial examination, including hematological determinations, was noncontributory. Thoracic roentgenogram revealed bronchopneumonia. The patient received 500 mg. of ristocetin every six hours and adjuvant supportive therapy for delirium tremens. The clinical picture remained unchanged for two days, with temperature fluctuating from 103 to 105 F. The dosage of ristocetin was then increased to 750 mg. every six hours. The patient began to improve on the fourth day of increased medication and three days later was free of all symptoms of respiratory tract infection. Two days subsequent to the increased dosage of ristocetin, a depression in white blood cells to 3600 cells/cu. mm. (granulocytopenia) was observed. After six days the count reverted to a normal level of 6700 white blood cells/cu. mm. No other toxic or side effects were noted. The patient made a complete and uneventful recovery.

#### SUMMARY AND CONCLUSION

Ristocetin was administered intravenously in the treatment of severe upper respiratory infections in 20 adult patients.

Daily dosages ranged from 750 mg. to 4 Gm., and duration of therapy averaged 4.8 days.

In 13 of 20 patients the results were excellent, with clinical response being evident within one to four days after institution of therapy. In 3 additional patients, there was some degree of improvement in pneumonic processes, superimposed on tuberculosis in 2 cases and on pulmonary neoplasm in 1. In all other cases, serious antecedent pathology undoubtedly influenced the negative or equivocal response to ristocetin therapy.

No local edema or phlebitis from intravenous administration was encountered.

Side reactions of a maculopapular rash in 1 patient and chills and fever in another were mild and transient.

Hematological changes occurring during treatment were 2 cases of thrombocytopenia and 1 of granulocytopenia. All symptoms abated with cessation of medication, and blood counts reverted to normal within seven days.

There was no evidence of adverse effects to medication in the renal, hepatic, pulmonary, or cardiovascular systems.

It is our belief that ristocetin is a safe and potent parenteral antibiotic for the treatment of respiratory tract infections, if used with proper technique and caution, together with frequent hematological studies.

#### BIBLIOGRAPHY

1. HWANG, K.; PRIMACK, N.; STEIN, R. J., AND RICHARDS, R. K.: Pharmacological and toxicological properties of ristocetins A and B. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 163.
2. GANGAROSA, E. J.; LANDERMAN, N. S.; ROSCH, P. J., AND HERNDON, E. G., JR.: Hematological complications arising during ristocetin therapy, *New England J. Med.* 259:156, July 24, 1958.
3. CALVY, G. L., AND SCHUMACHER, R. L.: Ristocetin and the *Staphylococcus*. Observations of effects and side effects in 17 cases of staphylococcal pneumonia, *J.A.M.A.* 167:1584, July 26, 1958.
4. SHIDLOVSKY, B. A.; PRIGOT, A., AND TURELL, R.: The influence of ristocetin on the aerobic intestinal flora of man. A preliminary report. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 199.
5. SHIDLOVSKY, B. A., AND PRIGOT, A.: Influence of combinations of ristocetin and neomycin on intestinal aerobic microflora. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 770.
6. ROMANSKY, M. J.; LIMSON, B. M., AND HAWKINS, J. E.: Ristocetin: A new antibiotic. Laboratory and clinical studies. Preliminary report. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 706.

Much has been written recently concerning hospital infections caused by *Staphylococcus*. Many reasons for this so-called epidemic have been given, and all the ways of combating it have been explored. During the past two years we have been fortunate in having the opportunity to observe clinically the effect of ristocetin in many staphylococcal infections, some of which were epidemic in nature in families, some of which were spontaneous in the hospital, and most of which were individual patients infected with the *Staphylococcus* organism.

Ristocetin (Spontin\*) has proved to be bactericidal and bacteriostatic, particularly for *Staphylococcus aureus*, which is often resistant to many other antibiotics. In all instances, in the successful use of ristocetin at this clinic, the organism proved to be sensitive in vitro. In our series, except in one instance, the drug was used only in the infections due to known gram-positive staphylococcal bacteria. The following are the types and number of infections in which the antibiotic has been used: osteomyelitis (foot, femur, radius, tibia), 7; cellulitis (face, elbow, foot), 5; septicemia, 3; pneumonia, 3; abscesses, 2; carbuncles and boils, recurrent, 2; postoperative wound infections, 5; and open fracture, 1.

## CASE REPORTS

*Patient 1.* J. Z., a 41 year old man, had chronic osteomyelitis following open reduction and internal fixation of the tibia and fibula. Because of extensive cellulitis, involvement of the soft tissues, and loss of bone due to the infection, he came under our care when a culture taken from him revealed hemolytic *Staphylococcus*. This organism was sensitive to 2.5  $\mu$ g. of ristocetin. From August 19 to 30, 1957, he was given 17,000 mg. of ristocetin intravenously, and from October 7 to 27, 1957, he was given 27,000 mg. The chronic edema and generalized cellulitis of his lower extremity improved on this therapy, but the leg is still draining, although the circumference of the leg has subsided and the general appearance is improved. The drainage at present is likely due to the screws and possibly some sequestrae still remaining in the leg. Ristocetin was used in two subsequent administrations because of a recurrence of the infection and for preoperative preparation.

*Patient 2.* L. S., a 56 year old man, was admitted to the hospital with an acute olecranon bursitis and cellulitis of the elbow, from which was cultured nonhemolytic *Staphylococcus*, sensitive to 2.5  $\mu$ g. of ristocetin. He had a diffusely swollen and acutely tender elbow when he was admitted to the hospital in August, 1957. For four days he was given procaine penicillin G and streptoduocin, and he developed a rash. From August 23 to 25 he was given 5000 mg. of ristocetin. During this period in the hospital, he was found to have diabetes. The infection of his elbow responded promptly and cleared completely a few days after his discharge from the hospital.

*Patient 3.* E. M., a 69 year old woman, developed osteomyelitis and diffuse infection of her right hip, following an open reduction and insertion of Smith-Petersen pin and a plate, from which was cultured hemolytic *Staphylococcus*, sensitive to 5.0  $\mu$ g. of ristocetin (tube dilutions). Early in the infection, from August 20 to 27, 1957, she was placed on streptomycin, but she did poorly, and from August 23 to September 1 she was given 18,500 mg. of ristocetin. The bacteria present proved also to be sensitive to novobiocin, and from September 4 to 11, she was placed

\* The trade name of Abbott Laboratories for ristocetin is Spontin.

on 250 mg. of novobiocin given orally four times a day. Her infection cleared rapidly, and we re-operated on her hip and inserted an Eicher replacement on September 4, 1957, with no post-operative complications. At the last follow-up, the hip was healed and she was walking well.

*Patient 4.* J. Z., a 63 year old man, developed cellulitis and gangrene of his right forearm, from which was cultured hemolytic *Staphylococcus*. Early in the disease, due to his critical condition, it was necessary to amputate the right arm as a lifesaving procedure. The arm had considerable necrotic tissue in the stump, and, after treatment with ristocetin, it is almost completely healed, although there is a small area still draining from the intramedullary canal of the radius that will have to be amputated at a slightly higher level.

*Patient 5.* M. K., a 40 year old man, fractured his left hip and had a severely comminuted fracture of the upper end of the femur in a badly deformed and osteoarthritic hip. He developed a postoperative infection from which hemolytic staphylococci were cultured, sensitive to 5.0  $\mu$ g. of ristocetin (tube dilution). During the course of this man's serious illness, he received the following drugs: from November 11 to 15, penicillin and streptomycin; from November 15 to 27, 250 mg. of novobiocin, four times daily; and November 27 to 30, 8000 mg. of ristocetin. This man is still under our care. He is still draining slightly and, probably, will until the pin and plate have been removed, but it was necessary to maintain stabilization of these fragments until adequate healing has taken place.

*Patient 6.* G. G., a 39 year old woman, was a patient of Gordner of Danville. She had had, for a long period of time, a *Streptococcus hemolyticus* septicemia. She was treated in another hospital, and we did not have a culture. However, we placed her on 3000 mg. of ristocetin daily. Her condition improved and her temperature dropped about a degree for the first few days, but she died on the fifth day. She received a total of approximately 15,000 mg. of ristocetin.

*Patient 7.* G. S., a 4 month old child with staphylococcic pneumonia. The bacteria were cultured only from her nose and throat. Also several members of the family had staphylococcal infections, including boils and abscesses. Phage tests have been run on the particular strain of *Staphylococcus*. This girl failed to respond after several days of other antibiotics, and on January 22, 1958, she was started on 25 mg./Kg. body weight of ristocetin. This drug was continued until February 3. Her temperature improved after the second day and continued normal until the time of discharge.

*Patient 8.* G. G., a 16 year old boy, developed an extensive cellulitis in the upper left cheek as the result of pinching pimples on the left eye and forehead. The entire left side of his face was swollen. He entered the hospital with a temperature of 100 F. The organism proved to be *Staph. aureus*, coagulase-positive. He was given ristocetin, 100 mg., twice daily for four days. The temperature was normal on the third day. He received 1,000,000 units of procaine penicillin G and 1 Gm. of streptomycin and recovered without complication.

*Patient 9.* W. B., a 51 year old man, developed an extensive cellulitis from a small pimple on his left cheek in 24 hours. His temperature rose to 103 F. The causative organism proved to be hemolytic *Staphylococcus*, coagulase-positive, with a positive blood culture and a diffuse glandular enlargement over the entire left neck. He received 1500 mg. of ristocetin twice daily for a total of 16,500 mg. His temperature rose to a height of 104 F., but on the fifth day returned to normal.

*Patient 10.* M. G., a 50 year old woman, had diabetes, with acute osteomyelitis and multiple small abscesses about her right knee due to *Staph. hemolyticus*, coagulase-positive. She received 1000 mg. of ristocetin twice daily for 10 days, during which period an incision and drainage with sequestrectomy was performed, with complete healing and recovery.

*Patient 11.* A. S. had recurrent abscesses of skin with an abscess of the right foot due to hemolytic *Staphylococcus*, coagulase-positive, which proved to be sensitive to ristocetin in vitro. He developed an extensive abscess of his right foot. He was given 1000 mg. of the drug twice daily for a total dosage of 20,000 mg. He made a complete recovery from the infection with no subsequent skin infections.

*Patient 12.* L. S., an 11 week old infant, developed a large abscess of the left thigh due to hemolytic *Staphylococcus* 52-42B-81. He received 25 mg./Kg. ristocetin for four and one-half days and had a complete subsidence of the abscess and symptoms,

TABLE I  
Summary of Sensitivity

Pt. no.	Bacterial culture	Penicillin	Chlortetracycline	Streptomycin	Oxytetracycline
1	<i>Staph. hemolyticus</i> , coagulase-positive	442	443		
2	<i>Staph. hemolyticus</i> , coagulase-positive	000	000		
3	<i>Staph. hemolyticus</i> , coagulase-positive	421	000	444	000
4	<i>Staph. hemolyticus</i> , coagulase-positive	444	444	444	444
5	<i>Staph. hemolyticus</i> , coagulase-positive	211		444	
6	<i>Str. hemolyticus</i>		NONE		
7	<i>Staph. hemolyticus</i> 52-42B-81	444	444	444	
8	<i>Staph. hemolyticus</i>	000		000	
9	<i>Staph. hemolyticus</i>	444	444	444	444
10	Nonhemolytic <i>Staphylococcus</i>	010	000	110	

*Patient 13.* C. L., a newborn baby, developed hemolytic staphylococcal (type 77) infection and rhinitis with wound infection following repair of harelip. She received ristocetin for eight days.

*Patient 14.* C. H. had a power mower accident involving the toes of his left foot, with subsequent cellulitis and osteomyelitis of the middle metatarsal. He was treated by débridement of the foot and ristocetin, 1000 mg. twice daily, with subsidence of temperature and acute cellulitis in four days.

*Patient 15.* E. E. T. was admitted to the hospital with a diagnosis of severe cellulitis of the face, with a temperature of 101 F. that rose each day. Temperature finally returned to normal on the seventh day. He received 14,000 units of ristocetin with no complications and a complete recovery.

*Patient 16.* L. H. had a postoperative left hip wound infection, with severe cellulitis and drainage. After ristocetin therapy, she was completely healed in one week.

#### LABORATORY STUDIES

Initially, we used dilutions of 20 to 1  $\mu\text{g.}/\text{ml.}$  ristocetin in tube dilutions in our sensitivity tests, but most of the later sensitivity tests were done by the disc method. Of all the infections due to *Staph. aureus* (gram-positive cocci) treated, novobiocin was the only other drug that could compare in the sensitivity tests with ristocetin. Most staphylococci were sensitive to ristocetin at the 2.5  $\mu\text{g.}/\text{ml.}$  level. (See table I.)

#### DISCUSSION

There has been considerable reluctance on the part of the physicians to use ristocetin without combining it with other drugs that have proved successful in the past. After the successful treatment of several of the previously mentioned patients, the author uses this drug almost daily at the present time. The average daily dosage has been 25 mg./Kg. body weight given in divided doses in 500 ml. of 5 per cent glucose, morning and evening. On one or two occasions in very severe infections, including staphylococcal septicemia, the daily dose was increased by 50 per cent, with 1 adult receiving as much as 1500 mg. twice daily. At first, the drug did not seem effective in mixed infections, for instance, on two occasions when the infection was mixed with *Pseudomonas*. The one death that occurred in this series was caused

TABLE I  
Studies in 10 Cases

Chloramphenicol	Erythromycin	Tetracycline	Novobiocin	Ristocetin	Sensitivity, to, $\mu\text{g.}/\text{ml.}$
420	321	444	Sens.	Sens.	2.5
421	000		Sens.	Sens.	
420	000	000	Sens.	Sens.	5
440	444	444	Sens.	Sens.	2.5
		444	Sens.	Sens.	
320	433		Sens.	Sens.	2.5
000	000	000		Sens.	3.1
441	443		Sens.	Sens.	
441	000	000	Sens.	Sens.	

by streptococcal septicemia. The patient had been treated in another institution without sensitivity tests and was moribund when the drug was administered.

It is my own personal opinion that the in vitro studies are not quite so accurate as the in vivo studies and that the intravenous administration of this drug perhaps makes it much more effective as far as the patient is concerned than the oral administration of many of the other drugs.

#### COMPLICATIONS

To date we have had no serious complications with the use of this drug. Leukopenia was evident in certain cases, which may or may not have been due to the effects of the drug. At least 2 of the patients experienced a sensation of tingling of the skin and a slight chill and became apprehensive because of these symptoms during the administration. In both instances the patients were quite ill, and subsequent injections failed to show this reaction. There was no skin, mouth, or other evidence of allergy to the drug, and in our series of cases we found no contraindication to its use.

#### SUMMARY AND CONCLUSIONS

Ristocetin was highly effective in the staphylococcal infections in which it has been administered in our institution. The intravenous administration of the drug may be considered a handicap and yet most of the patients to whom we administered the drug were sufficiently ill that they were nonambulatory. There have been no serious complications.

Ristocetin was used successfully in 30 patients with a variety of infections. This antibiotic proved effective in gram-positive cocci infections, being used chiefly in instances of *Staphylococcus hemolyticus*, coagulase-positive.

#### ACKNOWLEDGMENT

We are indebted to Abbott Laboratories for supplying ristocetin for this series of patients.

# Ristocetin in Adults and Children

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This study was undertaken to increase our knowledge of ristocetin\* in staphylococcal infections and to explore problems of dosage and toxicity in adults and children.

## CLINICAL MATERIAL

Adequate treatment with ristocetin was given to 11 adults and 6 children with infections due to resistant, coagulase-positive hemolytic *Staphylococcus aureus*. Another 4 patients received from one to three days of therapy and then succumbed to their predisposing illnesses—cerebral hemorrhage, congestive heart failure, and senility. One patient with gas gangrene received ristocetin seven hours before death without visible effect.

## CASE REPORTS

The 11 adults had all developed infections with coagulase-positive hemolytic staphylococci, which were resistant to at least one of the following antibiotics: penicillin, erythromycin, tetracycline, streptomycin, and chloramphenicol (see table I). They had all been treated without success with one or more of these antibiotics for five days to five months.

Ristocetin unequivocally saved the lives of patients 1 through 7 and, probably, of patient 10. In patient 11, it merely eliminated the staphylococci, which were replaced by other organisms. Patients 8 and 9 responded to ristocetin initially, which was then continued unnecessarily in patient 9 with the addition of chloramphenicol. However, both patients died with high fevers, which are presumed to be antibiotic fevers.

Patients 2, 4, 6, and 8 were all considered to be moribund before ristocetin was given; furthermore, patient 4 was an alcoholic with cirrhosis and a serum albumin of 2.1 Gm./100 ml. This patient recovered dramatically, in spite of only four days' therapy; ristocetin was then stopped because he was afebrile and had developed severe moniliasis following his 28 day exposure to antibiotics.

The 6 children all had resistant staphylococcal infections, which had been treated with other antibiotics for 13 to 60 days (see table II).

Ristocetin undoubtedly saved the lives of these 6 children, producing spectacular and dramatic results in all, except patient 16, who did not fully recover until a subperiosteal abscess was drained. Unfortunately, patient 14 died two weeks after cessation of ristocetin therapy from a fulminating enteritis. Patients 13 and 14 were moribund before ristocetin was started, with an extreme degree of emaciation in patient 14. It should be noted that patients 13, 15, 16, and 17 were given only three to four days therapy with ristocetin.

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\* The trade name of Abbott Laboratories for ristocetin is Spontin. The drug was furnished for this study through the courtesy of Abbott Laboratories.

TABLE I  
*Clinical Results in Staphylococcal Infections in Adults*

Pt. no.	Diagnosis	Ristocetin therapy				
		Before ristocetin therapy		Dose, Gm. every 6 hours		Result
		Condition	Treatment*		Days	
1	Chronic suppuration of hip joint	Emaciated, massive discharge of pus	P, E, T, C, 5 mo.	0.5 1.0	14 7	Good
2	Lung abscess, pyoderma	Moribund	P, E, T, S, 23 days	0.5	9	Recovered
3	Suppurative polybursitis	Emaciated, critical	P, 5 days	5.0† 1.0 1.0‡	0.5 8 17	Recovered
4	Pneumonia, cirrhosis	Moribund	P, T, C, 24 days	1.0§ 1.0	2 2	Recovered
5	Lung abscess, bilateral pneumonia	Critical	P, S, T, E, 16 days	0.75	11	Recovered
6	Relapsing pneumonia, septic thrombophlebitis	Moribund, emaciated	T, P, E, C, 49 days	0.75	11	Recovered
7	Carbuncle	Toxic	P, 5 days	0.5	7	Recovered
8	Pneumonia	Moribund	P, S, E, C, 7 days	0.5	10	Responded, died later
9	Pneumonia, suppurative arthritis	Critical	T, E, C, 5 days	0.5 1.5	14 3	Responded, died later
10	Huge ulcer on heel	Uncontrolled diabetes	P, T, 10 days	0.5	7	Healed, diabetes controlled
11	Chronic otitis media	Senile	P, 7 days	0.5	6	Fair

\* P = penicillin; E = erythromycin; T = tetracycline; C = chloramphenicol; S = streptomycin.

† Dose given in 12 hours.

‡ Dose given every 12 hours.

§ Dose given every 4 hours.

#### RESPONSE TO THERAPY

Although these staphylococcal infections did not respond to ristocetin immediately but only after an interval of several days, no patient continued to deteriorate after starting on ristocetin (see figs. 1-3).

As judged by toxicity and subjective indications, such as malaise and appetite, patients were often improved on the second day and were usually much better by the end of the fourth day. In 8 markedly febrile patients, however, the fever was unaffected for two days in all cases, for three days in 5 cases, and for four days in 2 cases. Fever was usually absent two days after the temperature started to fall. Normal temperatures were recorded on the fourth day of treatment in 3 cases, and between the fifth and seventh days in 5. Similarly, the main effect on pus was noted on the fourth and fifth days, except in patient 3, whose abscesses were distinctly better on the second day following the administration of the rather large dose of 7.0 Gm. in 24 hours (120 mg./Kg. body weight).

This delay in clinical response, especially as judged by the temperature chart, is expected in a bacteriostatic agent. It is, however, of considerable practical importance, since, in the somewhat panicky atmosphere that tends to surround febrile patients and their families, there is so frequently a readiness to switch from one antibiotic to another if the fever is not immediately controlled. Thus, in 3 cases, considerable argument took place before we were permitted to continue ristocetin

TABLE II  
Clinical Results in Staphylococcal Infections in Children

Patient no.	Age	Diagnosis	Before ristocetin therapy		Ristocetin therapy		
			Condition	Treatment*	Dose, daily, mg.	Days	Result
12	5 mo.	Empyema	Critical	P, T, C, 31 days	500 250	1 11	Recovered
13	21 mo.	Empyema	Moribund	P, E, T, C, 60 days	700 500	2 2	Recovered
14	7 wk.	Suppurative hip joint, pyoderma	Moribund	P, E, C, 36 days	125	8	Recovered
15	4 wk.	Diarrhea, Septic "cut-downs"	Critical	E, C, 13 days	150	3	Recovered
16	30 mo.	Osteomyelitis	Critical	C, E, 21 days	500 1.0 Gm.	1 3	Fair
17	5 wk.	Enteritis	Critical	E, C, 13 days	150	3	Recovered

\* P = penicillin; E = erythromycin; T = tetracycline; C = chloramphenicol.

therapy, simply because the patient's temperature was unaltered on the third day. In 1 case, because the temperature was unaffected on the third morning, streptomycin and isoniazid were added and continued for three days.

The delay in response does mean that with an organism resistant to ristocetin, vital days could be lost. Even though ristocetin-resistant staphylococci have not yet been demonstrated, sensitivity studies would therefore appear to be just as essential as with other antibiotics.

### DRAINAGE OF SUPPURATION

There were collections of pus in 7 patients. In 2 patients with empyema, closed drainage had already been instituted before ristocetin was started, and both recovered rapidly. In patient 14, a hip joint dislocated by suppuration was radically drained, and then ristocetin was started; a dramatic recovery ensued.

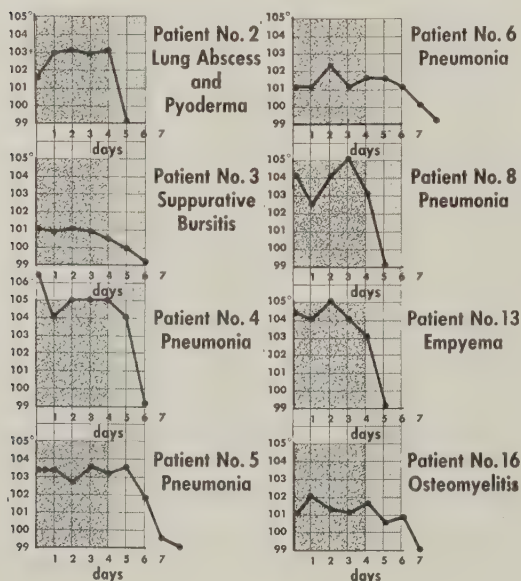
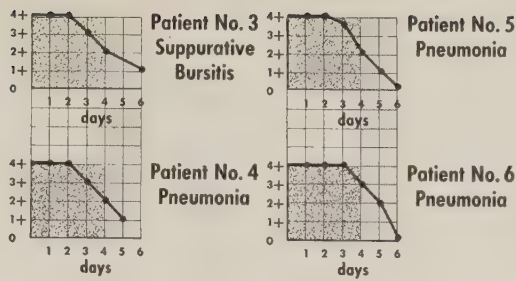


FIG. 1. The clinical response to ristocetin as judged by temperature is demonstrated.

FIG. 2. The clinical response to ristocetin as judged by toxicity is illustrated.



When effective surgical drainage was not instituted, the clinical response to ristocetin was much less impressive. In patient 1, an infected surgical reduction of a fractured femoral neck responded initially, as staphylococci were eliminated and the pus almost disappeared, but another organism then appeared; the hip finally healed after radical surgery and another course of ristocetin. In patient 3, the curious problem of suppurative polybursitis in a heroin addict was complicated by the patient's refusal to permit surgery. From time to time thick pus was aspirated from six bursae as they were affected. Ristocetin controlled the fever and toxicity, but he was not cured until an infected finger joint healed following surgery, which he finally permitted. In patient 16, ristocetin controlled the fever, but recovery was not achieved until after extensive surgery for osteomyelitis of the tibia.

Thus our experience indicates that ristocetin is not wholly effective when there is closed or inadequately drained suppuration, and that under such circumstances, normal surgical principles must be followed.

ADMINISTRATION OF RISTOCETIN

The instruction to give ristocetin by slow dilute intravenous infusion provoked numerous problems. Such an infusion in severely ill patients is difficult to maintain

FIG. 3. The clinical response to ristocetin as judged by purulent discharge is shown.

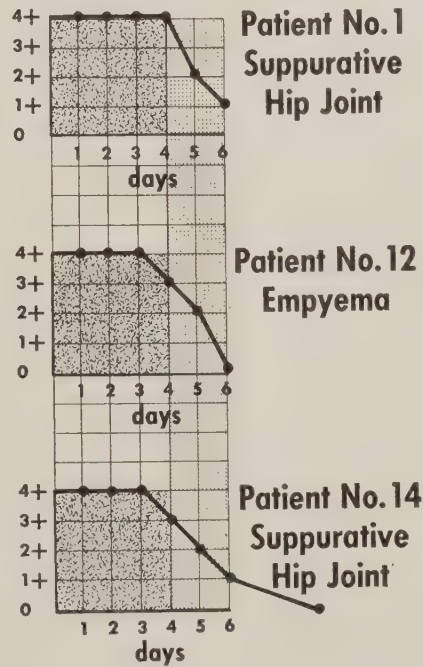


TABLE III  
*Administration of Ristocetin in Children*

Patient no.	Diagnosis	Age	Weight, lb., oz.	Dosage		Diluent, ml.	Days
				Daily, mg. every six hours	mg./Kg. body wt.		
12	Empyema	5 mo.	11, 14	62.5	50	500	11
13	Empyema	21 mo.	24, 0	187.5	70	500	2
				125	40	500	2
14	Suppurative hip joint	7 wk.	5, 5	125	52	75	8
15	Septic "cut-downs"	4 wk.	5, 10	150	53	20	3
16	Osteomyelitis	30 mo.	29, 0	500*	76	500	4
17	Enteritis	5 wk.	5, 3	150	63	20	3

\* Dosage administered every 12 hours.

for a number of days, and the water load involved can easily assume dangerous proportions. Also, when this equipment has to be tended twice every six hours while an infusion of ristocetin is started and stopped over a 45 minute period and, when this infusion appears to cause thrombophlebitis more readily than other infusions, the staff becomes somewhat resistant to using ristocetin therapy. In addition, we gradually gained the impression that faster infusions were more effective than slow ones.

*Rate and Concentration.* For these reasons, we gradually increased both the concentration and the rate of our infusions until, for example, 1 adult patient received 1.0 Gm. of ristocetin in 20 ml. of diluent over a period of two minutes twice a day for 17 days. There were no ill effects, local or systemic, and the staff was much better pleased with the therapy.

Similarly, with the children, 250 to 500 mg. in 500 ml. of diluent by slow infusion was the tedious initial procedure, whereas the last 2 children (patients 15 and 17) received intravenous injections of 150 mg. in 20 ml. of diluent in 5 to 10 minutes without ill effect (see table III).

*Dosage.* With adults, the usual dose was 50 mg./Kg. body weight daily in two to four divided doses; most patients thus received 2.0 to 4.0 Gm. daily. Ordinarily, a loading dose was not used, but patient 3 was given 5.0 Gm. in the first 12 hours, and two undrained abscesses were definitely improved the following day. Similarly, patient 4 responded rapidly to 6.0 Gm. (approximately 100 mg./Kg.) daily for the first two days.

With the children, the daily dosage was approximately 50 mg./Kg., except for 2 who received 70 mg./Kg.

*Frequency.* Ristocetin was administered every six hours to most adults. That this is not essential in every case is indicated by patient 3, with whom better therapeutic results were obtained when we switched to an injection of 1.0 Gm. every 12 hours. We also achieved excellent results with 3 children who received their daily total dose of ristocetin as a single intravenous injection.

#### TOXICITY

None of the patients developed skin rashes or hemorrhagic manifestations. The red blood cell count was depressed to 2,400,000 cells/cu. mm. in patient 9, but

chloramphenicol had also been given for 10 days prior to this blood count. The white cell count was depressed to 2700 cells/cu. mm. in patient 8, but this was seven days after discontinuing ristocetin and changing to chloramphenicol. The platelets were not affected in patients 8 and 9, nor were they affected in patients 1 and 3, who received the longest courses of therapy. Patient 1 developed an eosinophilia of 16 per cent, as well as anorexia and nausea with each course of ristocetin.

High spiking fever with shaking chills occurred in 3 patients. In patient 2, the high fever ceased abruptly when antibiotics were discontinued. In patient 9, who also had had chloramphenicol, the shaking chills were noted to be absent only during a 16 hour period when the infusion was discontinued; when the infusion was restarted, she again experienced a severe shaking chill with a temperature of 105 F. In patient 8, the evidence is more obtuse, and other causes for the fever have not been ruled out. Both patients 8 and 9 died; autopsy demonstrated toxic hepatitis in patient 9, no macroscopic cause of death in patient 8, and no infection in either. Antibiotic sensitivity may well have contributed to both deaths, but patient 8 was switched from ristocetin to chloramphenicol and erythromycin seven days before death, and patient 9 was also given chloramphenicol for 14 days and then novobiocin and oxytetracycline for two days before death, while the ristocetin was discontinued two days before death.

#### SUMMARY

1. Ristocetin completely controlled severe staphylococcal infections in 11 adults and 6 children who received adequate therapy.
2. With children, excellent results were obtained when the total daily dosage was given as a single intravenous injection in 20 ml. of diluent. With adults, two such injections daily often appeared adequate.
3. The response to ristocetin was often delayed until the fourth and fifth days.
4. Surgical drainage of closed suppuration was not eliminated by ristocetin.
5. Antibiotic fever occurred in 3 patients. The white count was depressed in one patient and the red count in another; both had also received chloramphenicol. No skin rashes occurred.

# Experience with Ristocetin in Staphylococcal Pneumonia

## Observations in 24 Patients

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Ristocetin\* has demonstrated its clinical effectiveness in the treatment of antibiotic-resistant, severe staphylococcal infections during a 22 month period at this hospital. A preliminary report on the treatment of staphylococcal pneumonia was presented by this department one year ago.<sup>2</sup>

This paper is an extension of earlier investigations.<sup>1,3</sup> Twenty-four patients with staphylococcal pneumonia have now received ristocetin, and in each case the causative organism was identified as coagulase-positive, hemolytic *Staphylococcus* (usually *Staphylococcus aureus*, although three were reported as *Staphylococcus albus*).

The 24 cases of staphylococcal pneumonia are outlined in table I, which includes the data on an additional patient with cervical chondritis, in whom extensive ristocetin blood level data have been obtained. The following case reports emphasize the severity of these infections and illustrate clinical response to ristocetin.

### CASE REPORTS

*Patient 5.* C. H., a 34 year old man, was admitted on October 16, 1957, because of fever, chills, hemoptysis, and left anterior chest pain of four days' duration. He was cyanotic and markedly tachypneic. Temperature was 103 F. and pulse rate, 120. Examination of the chest revealed bilateral pneumonitis, which was confirmed by chest roentgenogram (fig. 1). Sputum culture grew coagulase-positive, hemolytic, *Staph. aureus*. Because of abundant, thick, mucoid secretion, bronchoscopy was carried out at his bedside. Ristocetin was started immediately and tracheostomy performed. Temperature fell by lysis, and he was afebrile on the seventh hospital day. Ristocetin was continued for 15 days. Roentgenogram showed progressive clearing of pneumonia (fig. 2). Pulmonary function tests were normal four months later.

*Patient 25.* J. F., a 29 year old civilian technician, was the only survivor of the accidental explosion of several "Nike" missiles. He suffered 40 per cent second and third degree burns and, after six days of treatment, was transferred to this hospital. Fever of 104 to 105 F. did not remit with penicillin, streptomycin, and chloramphenicol. On the fourth hospital day, roentgenogram revealed bilateral pneumonitis (fig. 3). On the seventh hospital day operative débridement was carried out. The following day jaundice was present, temperature was 106 F., and sputum culture revealed coagulase-positive, hemolytic *Staph. aureus*. Ristocetin, 19.4 mg./Kg./day was begun intravenously and other antimicrobials discontinued. Temperature fell by lysis, and jaundice subsided. The patient had an uneventful recovery. Roentgenogram examination of the chest on the twenty-second hospital day was negative (fig. 4).

Seventeen patients received ristocetin following unsatisfactory response to other antimicrobial agents (table II). In 7 of these patients, early in this study, other antibiotic and chemotherapeutic agents were continued in conjunction with ristocetin. More recently, greater reliance has been placed on ristocetin alone.

### RESULTS

Ristocetin resulted in complete clearing of pneumonitis in 16 patients and satis-

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Opinions contained herein are those of the authors and do not necessarily reflect the view of the Navy Department.

\* The trade name of Abbott Laboratories for ristocetin is Spontin.

TABLE I  
*Ristocetin Treated Staphylococcal Infections*

Patient no.	Age, yr.	Diagnosis	Result
1° †	23	Leukemia; staphylococcal pneumonia	Clearing; died three months later
2° †	19	Poststaphylococcal pneumonia lung abscess	Complete clearing
3° †	21	Fractured spine and staphylococcal pneumonia	Complete clearing
4°	18	Postinfluenza staphylococcal pneumonia	Complete clearing
5°	34	Postinfluenza staphylococcal pneumonia	Complete clearing
6°	26	Postinfluenza staphylococcal pneumonia	Complete clearing
7	21	Influenzal pneumonia and staphylococcal superinfection	Died on fourth day of treatment
8°	18	Postinfluenza staphylococcal pneumonia	Complete clearing
9°	20	Postinfluenza staphylococcal pneumonia	Complete clearing
10° †	21	Heat stroke; staphylococcal pneumonia	Complete clearing
11	73	Postoperative metastatic bladder Carcinoma and staphylococcal pneumonia	Died on second day of treatment (four doses of ristocetin)
12°	63	Postoperative staphylococcal pneumonia	Complete clearing
13°	73	Postoperative staphylococcal pneumonia	Complete clearing
14°	41	Postoperative staphylococcal pneumonia	Sterile lung abscess, drained
15°	57	Carcinoma of mouth, staphylococcal pneumonia	Complete clearing; died three mo. later
16°	59	Cirrhosis, staphylococcal pneumonia	Clearing; died five days later of perforated ulcer
17°	23	Hodgkin's disease staphylococcal pneumonia and lung abscess	Died during therapy of widespread Hodgkin's disease
18°	22	Postinfluenza staphylococcal pneumonia and pericarditis	Complete clearing
19°	23	Postinfluenza staphylococcal lung abscess	Improvement, eventual surgery
20	72	Metastatic carcinoma and staphylococcal pneumonia	Died during first four hours (one dose of ristocetin)
21	66	Stroke and staphylococcal pneumonia	Complete clearing
22	23	Staphylococcal cervical chondritis	Gradual clearing
23	67	Metastatic carcinoma and staphylococcal pneumonia	Died during therapy of widespread malignancy; aspiration
24	58	Stroke and staphylococcal pneumonia	Clearing of first bout of pneumonia; died of stroke on fifth day second bout.
25	29	40 per cent burns and staphylococcal pneumonia	Complete clearing

° Patients referred to in reference 1.

† Previously reported in some detail in reference 2.

factory arrest of the infection in an additional 2 (table I). All but one of these (patient 2) were critically ill or moribund when ristocetin was begun. Surrounding infection cleared, and an abscess cavity diminished in size with ristocetin therapy in patient 19, who had a chronic staphylococcal infection.

Only 2 deaths (patients 7 and 11) were attributed to staphylococcal pneumonia; 4 (patients 17, 20, 23, and 24) died of other causes during therapy.

Not one instance of staphylococcal resistance to ristocetin was encountered.

#### METHODS OF ADMINISTRATION

Intravenous administration of ristocetin was accomplished by one of the following techniques: (1) direct, rapid injection of a 2 to 4 per cent solution in 10 to 15 minutes; (2) rapid drip (15 to 30 minutes) of 125 to 250 ml. of 0.4 to 1 per cent solution; and (3) slow drip (30 to 120 minutes) of 0.2 to 0.4 per cent solution, followed by slow infusion of 5 per cent glucose. Methods 1 and 2 were usually followed by a rapid infusion of 100 to 250 ml. of 5 per cent glucose.

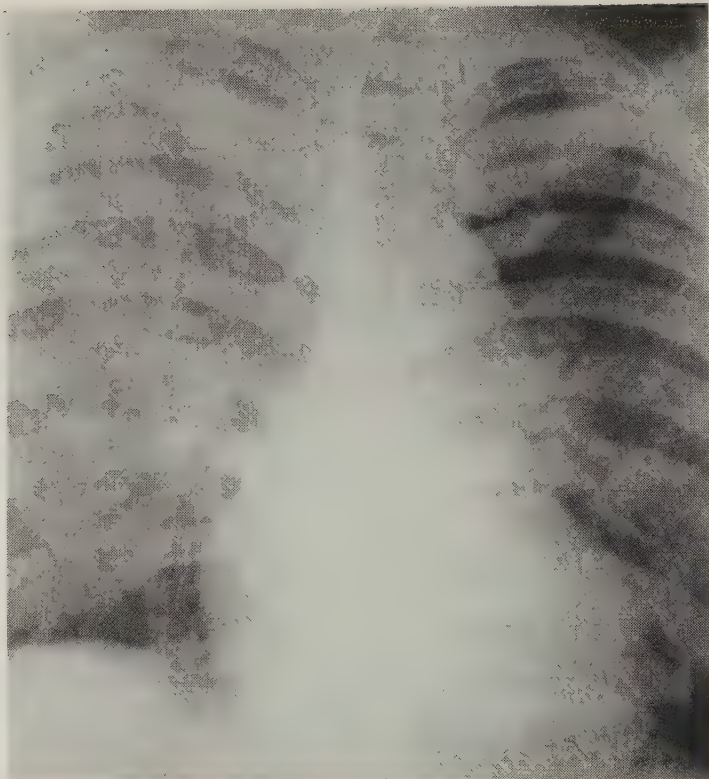


FIG. 1. Shown is a case of lobar pneumonia, right lower lobe, roentgenogram made on admission, October 16, 1957. (Case 5.)



FIG. 2. Complete resolution of the lobar pneumonia, right lower lobe, is shown in this roentgenogram made on November 4, 1957, after treatment with ristocetin. (Case 5.)

FIG. 3. This is roentgenogram made on the fourth day after admission, June 2, 1958, before treatment with ristocetin and showing lobar pneumonia in the left lower lobe. (Case 25.)



Phlebitis and venospasm were observed in three instances with method 3. Accidental infiltration was followed by phlebitis on one occasion (using method 1). Rapid drip (method 2), followed by a "flushing" infusion, has proved to be the safest and most convenient method.

FIG. 4. This roentgenogram taken on June 20, 1958, after treatment with ristocetin, shows complete resolution of the lobar pneumonia, left lower lobe. (Case 25.)

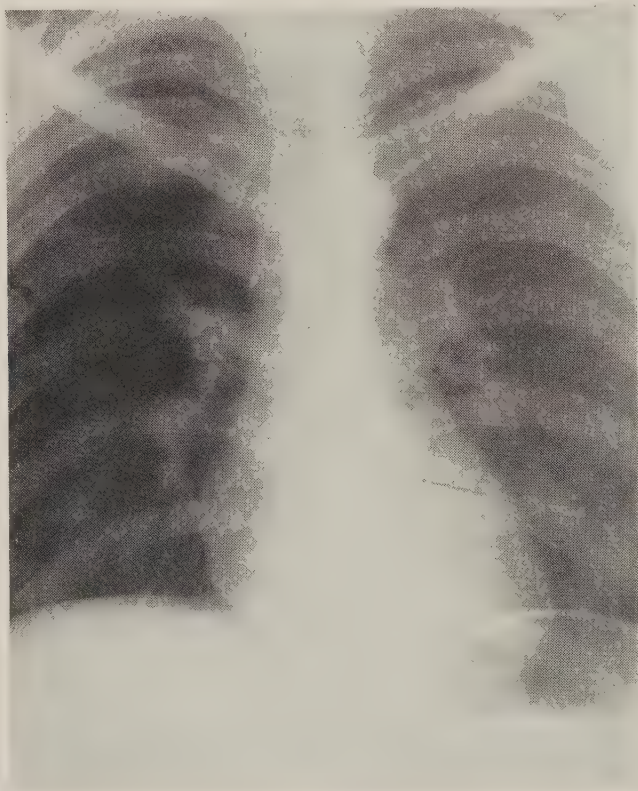


TABLE II  
*Other Antimicrobial Agents Used\**

Patient no.	Before ristocetin	With ristocetin
1	O,E,T,S	N,C
2	None	None
3	P,O,CT,C,SD,E,S,N	S,N,C
4	C	None
5	None	None
6	None	C added on sixth day
7	None	C added on third day
8	P,C	C
9	P	None
10	P,S,C	P,S,C
11	T,NE,N,P,S	C,N
12	None	None
13	None	C added on third day
14	SD,NE,C,P,S	C,S,P
15	P,S	None
16	P,S	CT,C
17	None	C,S,isoniazid,PAS
18	T,P,S	None
19	P,O,C,S,N	None
20	None	None
21	P	None
22	P,S	None
23	P,S	None
24	P,NY,N,T	None
25	P,S,C	None

\* P, penicillin; N, novobiocin; C, chloramphenicol; S, streptomycin or dihydrostreptomycin; T, tetracycline; O, oxytetracycline; NE, neomycin; E, erythromycin; CT, chlortetracycline; SD, sulfadiazine or other sulfonamides; NY, nystatin.

#### RISTOCETIN DOSAGE SCHEDULES

Dosage schedules (table III) varied widely. The drug was administered in two to four divided doses daily. The highest maintenance dose of ristocetin was 51.4 mg./Kg./day for 15 days (patient 16), and the lowest was 3.45 mg./Kg./day for six days (patient 10). At this time, patient 22 is entering his fifty-first day of therapy and has received a total of 76.5 Gm. of ristocetin, without any side effects.

The average daily dosage in this series was 1.8 Gm. or 24.6 mg./Kg. The average duration of therapy was 18.6 days. The shortest successful therapeutic course (patient 21) was six days at an average dosage of 15 mg./Kg./day.

#### RISTOCETIN BLOOD LEVELS

Ristocetin blood levels were obtained 8, 12, and 24 hours after dosage. These ranged from 3 to 9  $\mu$ g./ml., and accumulation did not appear. In patient 22, ristocetin blood levels obtained daily 12 hours after dosage of 9.7 mg./Kg. (19.4 mg./Kg./day) were as follows: 10  $\mu$ g./ml. on 20 occasions and 5  $\mu$ g./ml. on 22 occasions.

A single sample (patient 15) taken 12 hours after the initial dosage of 14.2 mg./Kg. revealed a blood level of 4.2  $\mu$ g./ml. Eight samples obtained on the tenth through fifteenth days of therapy revealed a range from 4.4 to 6.6  $\mu$ g./ml. Each of these was obtained 12 hours after a dosage of 7.1 mg./Kg.

TABLE III  
*Ristocetin Dosage Schedules*

Pt. no.	Initial		Maintenance						Average		
	mg./Kg.	Days	mg./Kg.	Days	mg./Kg.	Days	mg./Kg.	Days	Gm./day	mg./Kg.	Total days
1	13.8	32							1.0	13.8	32
2	16.9	8	8.5	7	16.9	18			0.9	15.2	33
3	16.9	13							1.0	16.9	13
4	*		30.0	10					3.0	30.0	10
5	41.4	8	27.6	4	13.8	3			2.33	31.9	15
6	*		41.4	1	27.6	2	41.4	5			
			27.6	1	20.7	4	13.8	2	2.8	38.3	15
7	35.5	1	25.9	2½					2.2	28.4	3½
8	38.8	9	19.4	5	12.9	4			2.14	27.6	18
9	*		41.4	5	27.6	4	13.8	4	2.1	28.8	13
10	41.4	4	13.8	3	6.9	6	3.45	6	1.02	14	19
11			27.6	†							
12	*		43.8	1	21.9	2	14.6	8			
			7.3	4					1.22	17.9	18
13	38.8	11							3.0	38.8	11
14	24.2	3	33.8	23					1.9	32.9	26
15	†		21.3	5	14.2	20	7.1	3	1.0	14.2	28
16	38.7	1	51.4	15					3.93	50.8	16
17	*		36.6	1	24.4	18			2.05	25.0	19
18	27.6	16	13.8	7					1.69	23.1	23
19	18.3	13							1.5	18.3	13
20	†										
21	*		19.4	2	12.9	2			1.16	15	6
22	19.4	50 (cont.)							1.5	19.4	50 (cont.)
23	33	1	22	5	44	2			1.3	28.8	8
24	33	7	22	5					1.25	27.5	12
25	25.9	2	19.4	12					1.57	20.2	14

\* Single dose of 750 mg.

† Single dose of 500 mg.

‡ Only four doses administered.

Ristocetin blood levels obtained in patient 12 are outlined in table IV. Blood levels obtained 8 to 24 hours after these low doses of ristocetin exceeded the minimal inhibitory concentration for the organisms within the "sensitivity spectrum" of ristocetin.

#### HEMATOLOGY

No purpura or abnormal bleeding was observed in the entire series. Normal serial platelet counts were obtained in 9 patients. On the third day of treatment with 41.4 mg./Kg./day, patient 10 had a platelet count of 77,000 cells/cu. mm. He had suffered a severe heat stroke and was moribund. Mild azotemia was present on admission, but abated during ristocetin therapy. Following reduction in dosage, blood smears showed a normal amount of platelet material. This patient remains well one year after treatment.

Patient 6 had a platelet count of 82,000 cells/cu. mm. after nine days of therapy at a dosage level of 41.4 mg./Kg. Five days later, following reduction to 20.7 mg./Kg./day, his platelet count rebounded to 1,100,000 cells/cu. mm. and was normal thereafter.

Except for the leukocytosis of infection, no abnormalities of white blood cell count or differential were observed. Slight decreases in hemoglobin and hematocrit were observed in 4 patients. A similar anemia has been observed in 5 patients with staphylococcal pneumonia who did not receive ristocetin.

TABLE IV  
*Ristocetin Blood Levels (Patient 12)*

Day of treatment	Previous dosage, mg./Kg.	Interval, hours	Blood level, μg./ml.
½	7.3	8	6.1
1	7.3	12	5.9
4	7.3	12	5.9
6	7.3	12	6.5
9	7.3	24	4.5
10	7.3	24	6.6
11	7.3	24	2.9
12	7.3	24	2.6

#### VENOUS SPASM AND PHLEBITIS

Local venous spasm, which made injection of ristocetin both painful and difficult, was observed in 2 patients. Two other patients developed phlebitis without thrombosis. One of these, an intern (patient 6), developed phlebitis after an unsuccessful attempt at self-administration. The others had indwelling venous cut-down catheters and received ristocetin by slow infusion in dosage exceeding 38 mg./Kg./day. Venous reactions did not recur in 2 patients whose treatment was continued at reduced dosage.

#### DRUG FEVER

Fever, presumably related to ristocetin administration, was observed in 3 patients. This began in patient 4 at a dosage of 30 mg./Kg./day, ranged between 99 and 101 F., and disappeared on withdrawal of ristocetin. The fever observed in patient 6 coincided with the occurrence of phlebitis, but abated with drug withdrawal.

Persistent fever observed in patient 3, at a dosage level of 16.9 mg./Kg./day, was probably due to ristocetin, although other drugs were concurrently administered.

#### SUMMARY OF SIDE EFFECTS

No dermatitis or other cutaneous changes were observed in the total experience. Multiple laboratory examinations were obtained on these patients, and there was no observed change in macroscopic or microscopic examination of the urine, blood urea nitrogen, blood sugar, serum proteins, or liver function (table V).

TABLE V  
*Side Effects*

Side effect	Patient no.	Dosage on onset	Comment
Platelet depression	6	41.4	Rebound with dosage reduction
Platelet depression	10	41.4	Well one year later
Venous spasm	9	41.4	Slow infusion cut down
Venous spasm	13	38.8	Slow infusion cut down
Phlebitis	16	51.4	Slow infusion cut down
Phlebitis	6	41.4	Infiltration
Drug fever	4	30.0	Abated with withdrawal of ristocetin
Probable drug fever	6	41.4	Abated with withdrawal of ristocetin
	3	16.9	Abated with withdrawal of ristocetin

Eight of 9 recorded side effects occurring at dosages exceeding 29 mg./Kg./day. It is our impression that platelet depression is directly related to dosage. Phlebitis and venous spasm are probably related to the method and duration of administration, although high daily dosage may also be a factor. Two of the 4 patients with venous reactions were continued on ristocetin at reduced dosage without recurrence.

#### SUMMARY AND CONCLUSIONS

1. Ristocetin was used in the treatment of 24 patients with staphylococcal pneumonia, 17 of whom had failed to respond to previously administered antibiotics.

2. Complete clearing of pneumonitis was obtained in 16 patients, and significant improvement occurred in 2 others. Two patients died of pneumonia; 4 others succumbed to other lethal diseases.

3. Rapid intravenous drip was the most satisfactory method of administration.

4. Initial dosages varied widely but averaged 24.6 mg./Kg./day. The recommended dosage is 25 mg./Kg./day.

5. Serial ristocetin blood levels were obtained 12 to 24 hours after drug administration in 3 patients and exceeded the minimal inhibitory concentration for organisms sensitive to ristocetin.

6. All but 1 of 9 observed side effects occurring at dosages higher than 29 mg./Kg./day.

7. No instance of bacterial resistance to ristocetin was encountered.

8. The rapid clinical response achieved, with toxicity minimized at the dosage employed, attests to the potency of ristocetin.

#### ACKNOWLEDGMENT

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#### BIBLIOGRAPHY

1. CALVY, G. L., AND SCHUMACHER, L. R.: Ristocetin and the *Staphylococcus*: Observations of effects and side effects in seventeen cases of staphylococcic pneumonia, J.A.M.A. 167:1584, July 26, 1958.
2. TAYLOR, D. E.; SCHUMACHER, L. R., AND CALVY, G. L.: Ristocetin in the treatment of antibiotic-resistant staphylococcal pneumonia. In: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, pp. 180-186.
3. SCHUMACHER, L. R.; TAYLOR, D. E.; HOCHMAN, R. I., AND CALVY, G. L.: Clinical studies with ristocetin in the treatment of hospital acquired staphylococcal pneumonia, Clin. Research 6:36, Jan., 1958.

# Pyridoxine and Its Relation to Cycloserine Neurotoxicity

## A Pharmacological and Clinical Study

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The neurotoxicity that sometimes occurs from large doses of cycloserine\* can now be controlled by pyridoxine.<sup>1-3</sup> † The fear engendered by stressing this toxicity has slowed the acceptance of cycloserine for use in chronic, resistant cases of tuberculosis,<sup>4-6</sup> despite the fact that its use has reduced the high death rate in such cases, now treated with other drugs.<sup>7</sup>

There is strong evidence that cycloserine used alone is effective therapy in tuberculosis and that it retains its effectiveness against isoniazid- and/or streptomycin-resistant bacilli.<sup>2, 8, 20</sup> Used alone, it has proved as good and as safe as either isoniazid alone or streptomycin alone, while bacillary resistance to it develops much more slowly and to a lesser degree.<sup>24</sup> The lifesaving properties of cycloserine in drug-resistant tuberculosis have led to a search for ways to lessen its neurotoxic effects, and, in 1957, we reported complete prevention of severe toxicity from a daily dose of 2.0 Gm. of cycloserine, using .300 mg./day of pyridoxine hydrochloride.<sup>2, 3</sup>

Barclay et al<sup>9</sup> used phenobarbital and diphenylhydantoin in a group of 14 far-advanced, cavitary, bilateral, pulmonary tuberculosis cases, resistant to isoniazid and streptomycin, to control successfully the toxicity from a large dose of cycloserine (27 mg./Kg./day). The clinical response was excellent.

Cycloserine owes its antituberculous effects in man to its ready absorption and the attainment of plasma and tissue levels well above those that are necessary for bacillary inhibition in vitro. In addition, its small molecule and water solubility allow easy penetration into tuberculous tissues<sup>10</sup> and the monocytic cells containing the bacilli. Occasionally, neurotoxicity occurs in the form of hyperreflexia, tremors, psychotic disturbances, and convulsions, reminiscent of the toxicity from large doses of isoniazid.<sup>11-13</sup>

The search for a dietary factor to account for the neurotoxicity of cycloserine stemmed from the fact that tuberculosis is a toxic and debilitating disease, associated with chronic malnutrition. It was known that convulsive movements and hyperirritability occurred in many species of animals on a B<sub>6</sub> deficient diet and that infants have had convulsions when fed a formula accidentally deficient in B<sub>6</sub>.<sup>14-16</sup> However, pyridoxine deficiency does not lead to convulsions in adult subjects. Such diets result in neuritis and dermatological lesions, which do not occur during prolonged therapy with cycloserine. By a process of elimination, we had determined that niacin, riboflavin, and thiamine, singly in large doses or combined in a complete vitamin supplementary mixture, did not influence the incidence of cycloserine neurotoxicity. Pyridoxine hydrochloride was found to do so in daily doses of 50 mg. or more.

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\* The trade name of Eli Lilly & Co. for cycloserine is Seromycin.

† The trade name of Eli Lilly & Co. for pyridoxine is Hexa-Betalin.

TABLE I  
*Neurotoxic Reactions from Cycloserine*

Gm. daily	No. patients	Convulsions		Pyridoxine added
		No.	Per cent	
0.5	160	1	0.6	4*
1.0	119	2	1.7	1*
1.5	41	4†	7.8	11*
2.0	27	0	0	27
Total	347	7	Av. 2.0	43

\* Supplemented after onset of symptoms.

† One patient was an epileptic, who became worse.

#### SALUTARY EFFECTS

A summary of the occurrence of neurotoxic symptoms from doses of 1.0 Gm./day or more has been previously reported.<sup>24</sup> The incidence of convulsions reported by Murray<sup>4</sup> and by Storey et al<sup>15</sup> was greater than that reported by others, but this may have been due to the small number of their cases. Our incidence of 3.5 per cent (compared to the 8 per cent reported by Storey<sup>20</sup>) may be accounted for, in part, on the basis of the daily dose being given four times, rather than two times, daily, resulting in lower peak plasma levels.

The protective action of pyridoxine against the cycloserine neurotoxicity is shown in tables I and II. The 27 far-advanced, cavitary, drug-resistant cases of pulmonary tuberculosis were treated with 2.0 Gm./day of cycloserine and 300 mg./day of pyridoxine hydrochloride, for the purpose of determining the protective effect of the pyridoxine as well as the antituberculous effects of the cycloserine in such cases. The patients presented a history of from 2 to 27 years (average 6.8) of continuous and progressive ill health. Each patient was thoroughly examined, especially in regard to the central nervous system, and questioned about psychic background. A routine roentgenogram of the chest was made and sputum collected, examined, and cultured for bacillary content. Other routine studies included hemograms, liver and kidney function tests, and estimates of well-being, appetite, degree of cough, amount of sputum, and sleeping habits.

The data in tables III and IV show the pretreatment condition of these patients, i.e., long-standing, far-advanced, and fibrocavitary and destructive lesions of the lungs. All but 3 had had a persistently positive sputum for years.

#### DOSAGE OF CYCLOSERINE

Because our objective was the prevention of toxic symptoms that might result

TABLE II  
*Incidence of Reactions in 27 Patients Receiving Cycloserine\**

Type of reaction	Incidence†	Average plasma level, $\mu\text{g.}/\text{ml.}$
Hyperreflexia	6	53.7
Mental confusion	6	47.3
Dizziness or drowsiness	8	44.4
Convulsions	0	—
Total	20	49.0

\* Dosage was 23 to 54 mg./Kg./day.

† Seven patients had more than one type of reaction. A total of 13 patients reacted.

TABLE III  
Statistical Data on 27 Patients

Sex	No. of pt.	Age range, yr.	Caucasian	Negro	Puerto Rican	Chinese
F	4	24-35	0	2	2	0
M	23	23-65	6	10	5	2
Total	27	46 (av.)	6	12	7	2

from large doses of cycloserine, all other antituberculous therapy was discontinued. At the outset of these observations, regardless of body weight (tables IV and VI), each patient received an oral dose of 2.0 Gm./day of cycloserine, averaging 31 mg./Kg. (22 to 51 mg./Kg.). This was given in two capsules, each containing 0.25 Gm. of cycloserine four times a day, with a 10 to 12 hour interval between the evening and morning doses.

Blood was collected for the determination of plasma levels of cycloserine just prior to the morning dose, unless otherwise designated. Concomitantly, 300 mg./day of pyridoxine hydrochloride was administered in three equal doses.

Tables II and V to VII summarize the data on the toxic manifestations and relate them to daily dosage and plasma levels of cycloserine. The salutary clinical responses, to be reported in detail elsewhere, must be assessed on the basis of the severity of the illness at the start of cycloserine therapy.

There was a prompt increase in appetite and gain in weight in 21 of the 27 patients, although every one of them had been stationary or losing weight prior to cycloserine therapy. Bacteriologically, the results were very encouraging. Within 6 to 11 weeks of treatment, most of the 24 sputum-positive cases became negative on smear and 11 on culture as well. There was definite roentgenographic evidence of improvement in 8 of the 27 patients, although the initial fibrosis made it difficult to appraise accurately the degree of change. Table VI shows the clinical improvement as an index of survival and rehabilitation, resulting from the cycloserine therapy in these 27 otherwise doomed and hopeless cases of tuberculosis.

#### REACTIONS TO CYCLOSERINE THERAPY

Four of the 27 patients reacted with rises in temperature to 100, 101, 102, and 103.6 F., respectively, lasting for one to six days. Fever reactions had been reported following the administration of cycloserine in active cases of tuberculosis.<sup>17</sup> Because none of these subjects had fever prior to the institution of cycloserine therapy, it is surmised that the fever was related to the administration of cycloserine. However, it is unlikely that the fever was due to the drug per se, because it occurred only at the onset of therapy, was evanescent, and did not appear later, despite continuous administration of cycloserine for periods of one year or more. The fever was prob-

TABLE IV  
Initial Clinical Status of 27 Patients

Sputum	Roentgenogram*	Clinical status	Bed status	Weight, lb.	Years of illness
Pos., 24	Far-advanced, 25†	Prog., 12	Bed, 10	81-196	2-27
Neg., 3	Moderately advanced, 2	Stat., 15	Ambulatory, 17	Av. 132	Av. 6.8

\* All cases were fibrocavitary.

† Includes the 3 sputum-negative cases.

TABLE V  
*Relation of Dose and Plasma Level to Incidence of Side Effects*

Type of patient	No.	Average age, yr.	Average daily dose, mg./Kg.	Average plasma level, $\mu$ g./ml.
Total Patients	27	46.0	34.9	40
Reactors	13	46.5	37.7	49
Nonreactors	14	45.5	32.6	32
Reactors minus nonreactors			5.1*	17†
Negro Patients				
Reactors	9	46.0	35.0	47
Nonreactors	3	38.0	30.0	29
Reactors minus nonreactors			4.7*	18†

\* Standard error of mean, 2.2; difference is not statistically significant.

† Standard error of mean, 4.2; difference is statistically significant.

ably a Herxheimer type of reaction, such as occurs from penicillin during the treatment of early syphilis.<sup>18,19</sup>

Untoward reactions were observed in 13 of the 27 patients, but in no case was it necessary to permanently discontinue therapy. Slight and temporary mental confusion, dizziness, tremors, and hyperreflexia were the most frequent symptoms (table II). In 1 patient with persistent hyperreflexia and mental confusion, the dosage of cycloserine was halved to 1.0 Gm./day. The symptoms cleared promptly and did not recur upon resumption of the full dose one week later.

In 3 other patients with severe tremors, the cycloserine was discontinued for two to three days and then resumed. One of these developed symptoms again about six months later, and the drug was discontinued for seven days. The full dose was then resumed, without further toxic effect. In the other 2 patients, the symptoms cleared promptly upon discontinuance of the cycloserine for a few days and did not recur, despite the reinstitution of the full dose.

There were no convulsions. It is possible but unlikely that convulsions would not have occurred, even without the prophylactic administration of pyridoxine hydrochloride. Convulsions are but infrequently elicited by large doses of cycloserine, as illustrated by comparing data from several hospitals.<sup>24</sup> Barclay et al<sup>9</sup> reported only one convulsion in a series of 14 patients treated with 27 mg./Kg./day of cycloserine.

TABLE VI  
*Present Status of 27 Patients*

Weeks of therapy	Hospitalized		Nonhospitalized		
	Bed	Ambulatory	Discharged to outpatient dept.	Signed out* to outpatient dept.	Signed out, lost
0	10	17	—	—	—
6-12	0	1	0	0	3
12-24	0	3	1	4†	3
24-36	0	8‡	4	0	0
Total	0	12	5	4	6

\* Signed out against advice.

† One of these patients died from a nontuberculous cause four weeks after leaving hospital.

‡ One of these patients died from a sudden hemorrhage, who had been consistently sputum positive and underweight.

TABLE VII  
*Relation of Body Weight to Reactions*

Type of patient	No.	Weight difference from ideal							
		Sex		Normal		Overweight		Underweight	
		M	F	No.	No.	Lb.	No.	Per cent	Lb.
Reactor	13	11	2	1	2	12 (9-15)	10	76	25 ( 9-39)
Nonreactor	14	12	2	3	4	21 (9-31)	7	50	28 (18-43)
Total	27	23	4	4	6	18 (9-31)	17	63	Av. 26 ( 9-43)

However, because his subjects received central nervous system depressants as soon as any symptoms appeared, the further occurrence of convulsions may have been masked. That pyridoxine was effective in reducing the incidence and severity of the toxicity of cycloserine in our series is supported by the fact that all types of neurotoxic symptoms occurred in only about 40 per cent, compared to more than 80 per cent in Barclay's patients.

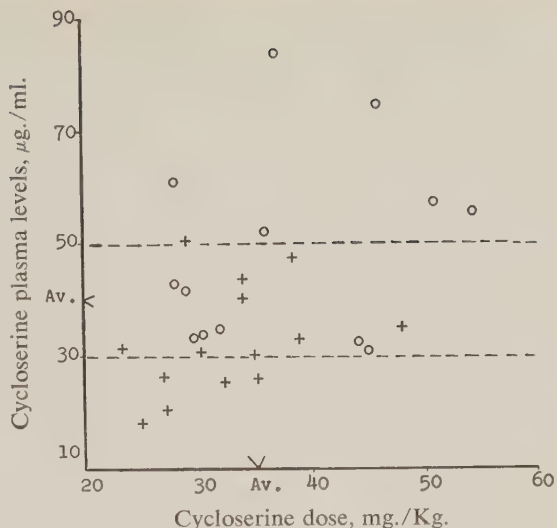
There were 13 patients who reacted, 7 of whom had two types of reactions each (table II). All but two of the more severe reactions occurred during the first six weeks of therapy. Drowsiness and dizziness occurred during the first few days and were evanescent. One case of mental confusion occurred after eight weeks of therapy, and 1 patient, who had temporary mental confusion during the first week, developed hyperreflexia during the twenty-fifth week of therapy.

The data in tables II and V reveal little evidence of correlation between the oral dose of cycloserine and the number and types of reactions. The nonreactors were given an average dose of 32.5 mg./Kg./day, compared to 37.5 mg./Kg./day for the reactors, but the 5 mg. difference was not statistically significant (table V). On the other hand, there was evident correlation between the plasma level of cycloserine and the occurrence of reactions. The nonreactors averaged 32  $\mu$ g./ml., compared to 49  $\mu$ g./ml. for the reactors. The difference of 17  $\mu$ g./ml. was statistically highly significant.

Further analysis of the data revealed little relationship between the occurrence of the two most disturbing types of untoward symptoms—hyperreflexia and mental confusion. It is surmised that these two distinct types of reactions occur independently in subjects treated with cycloserine and should not be considered as part of an over-all toxicity phenomenon. Of 10 patients who had one or the other of these reactions, only 2 had both, but not simultaneously. One of these showed hyperreflexia during the first week of treatment and mental confusion during the eighth week. The other showed mental confusion the first week. Therapy was discontinued for three days, and the patient recovered completely. During the twenty-fifth week of cycloserine therapy, this patient developed hyperreflexia. Therapy was discontinued for one week, with prompt and complete recovery. Following reinstitution of therapy, this patient remained free of symptoms, despite consistently high cycloserine plasma levels of from 58 to 104  $\mu$ g./ml. In one test the plasma level rose to 111  $\mu$ g./ml. three hours after the morning dose of 0.5 Gm., without evidence of symptoms.

The racial origin of these 27 patients may indicate some relationship to the occurrence of toxic symptoms, although the number is too small to be reliable. Tables III

FIG. 1. The relation of dose to plasma levels is illustrated. O, reactors; +, nonreactors.



and V show that there were 12 Negroes in this series, 9 or 75 per cent of whom reacted, compared to 4 or 27 per cent of the 15 other patients. The 9 reacting Negro patients showed slightly lower plasma levels than the 4 other reacting patients. It is possible that the higher incidence of symptoms in the Negro patients was due to a higher central nervous system sensitivity (table VI).

It was likely that the toxic manifestations were related to the degree of malnutrition present in these subjects, a factor that would correlate with the effectiveness of pyridoxine hydrochloride in controlling the symptoms. Table VII shows an analysis of reactions as related to the initial body weight and its relation to the "ideal" weight, obtained from actuarial tables for Caucasians. Again, the number of patients is too small to draw reliable conclusions. Ten, or 76 per cent, of the 13 reacting subjects were underweight, compared to 7, or 50 per cent, of the 14 nonreactors. Compared to the 63 per cent of all subjects who were underweight, the differences between the reactors and nonreactors are not significant.

However, three conclusions can be readily drawn from the data: (1) The oral dose cannot be taken as a reliable index of the eventual plasma level of cycloserine. As shown in figure 1, there is a rising base line as the oral dose is increased, yet above this line there is no correlation; (2) the occurrence of reactions to cycloserine was only casually related to plasma levels (fig. 1). In the group of 15 patients with levels between 30 and 50  $\mu\text{g./ml.}$ , 7 reacted and 8 did not. The 6 patients with levels greater than 50  $\mu\text{g./ml.}$  reacted, while the 6 with levels less than 30  $\mu\text{g./ml.}$  did not react; and (3) neither the oral dose nor the plasma concentration of cycloserine will forecast the occurrence of toxic symptoms. The symptoms shown in table II occurred only temporarily, and for short periods, but the dose and plasma levels of cycloserine remained high. The plasma levels were maintained well within the range peculiar to each patient, and there was no evidence that long-term, daily administration of cycloserine in any way influenced the maintenance of these levels. Cycloserine plasma levels were not especially high during the toxic symptoms.

Liver and kidney function tests, urinalyses, and complete hemograms showed no evidence of tissue damage. Tryptophane load tests did not increase the excretion of xanthurenic acid in the urine, indicating that this pathway of  $B_6$  utilization was not altered. Despite year-long, daily, oral administration of cycloserine, there was no overgrowth of *Monilia*, yeasts, fungi, or other organisms in the intestinal tract

or about the mouth and anal regions. A sense of well-being, bordering on euphoria, was reported by some patients, especially at the outset of treatment.

## EXPERIMENTAL

*Effects of Cycloserine on Pyridoxine-Depleted Rats.* Because convulsions may be elicited in animals on a diet devoid of B<sub>6</sub>, the following experiments were undertaken.

The young rats used in this study were of a uniform Hollowbrook Farms strain. A synthetic diet, complete in all other respects but devoid of vitamin B<sub>6</sub>,<sup>21</sup> was supplied. Groups of 10 or more animals, weighing between 50 and 65 Gm., were used in all experiments. The animals were weighed daily and daily records kept of the amount of food and water taken. In the first series of experiments on 20 rats, single, weekly, subcutaneous injections of cycloserine, 1.0 Gm./Kg., were made. On the following day, two subgroups of 10 animals each were formed, one of which was continued on the B<sub>6</sub> enriched laboratory pellet diet. The other 10 animals received the B<sub>6</sub> deficient synthetic diet. At approximately weekly intervals thereafter, all 20 animals received provocative injections of 1.0 Gm./Kg. of cycloserine.

Definite signs of B<sub>6</sub> deficiency were evident at about the twentieth day. These became progressively worse; 2 rats died on the thirty-fourth day, 3 on the thirty-eighth day, and 8 had died by the forty-second day. The remaining 2 rats were returned to a complete diet, with prompt and complete recovery.

Some of the B<sub>6</sub> deficient rats hyper-reacted to jarring of the cage, to loud noises, or to being touched. As the experiment progressed, the responses to touch and sound became still more exaggerated. Near the time of death, some of the rats manifested sudden, spasmodic movements resembling momentary convulsive spasms, but no frank convulsions occurred.

The periodic administration of the large dose of cycloserine did not further

TABLE VIII

*Effects of Cycloserine\* on 10 Normal and 10 Pyridoxine Deficient Rats*

Cycloserine injections	Group	Diet	No.	Days fed	Av. weight, Gm.	Symptoms	
						From B <sub>6</sub> deficiency	From cycloserine injection†
1	A	Con.	10	0	60.2	None	None
	B	—B <sub>6</sub>	10	0	60.7	None	None
2	A	Con.	10	21	95.9		None
	B	—B <sub>6</sub>	10	21	68.7	Wt. loss	None
3	A	Con.	10	28	110.1		None
	B	—B <sub>6</sub>	10	28	63.9	Swollen, red paws	None
4	A	Con.	10	35	115.8		None
	B	—B <sub>6</sub>	8‡	35	57.0	Nasal discharge	None
5	A	Con.	10	40	128.2		None
	B	—B <sub>6</sub>	5§	40	47.6	Dermatitis	None

\* Dosage was 1.0 Gm./Kg., given subcutaneously.

† Only the occurrence of severe symptoms of neurotoxicity or convulsive seizures is shown in this column.

‡ Two rats died on the thirty-fourth day.

§ Three rats died on the thirty-eighth day.

increase the central irritability (table VIII). If cycloserine had elicited a central convulsive action, it would have become manifest as an exaggeration of the convulsive state induced by the lack of vitamin B<sub>6</sub>. It may be concluded that cycloserine does not stimulate directly those centers in the brain that induce convulsions, whether these centers are normal or hypersensitive.

If cycloserine further depletes the body of B<sub>6</sub> activity, as with isoniazid<sup>22</sup> or deoxypyridoxine,<sup>23</sup> it would be more likely to do so if fed daily in large doses over prolonged periods, in simulation of its clinical use. To test this hypothesis, 24 rats were fed the B<sub>6</sub> deficient diet, in a second series. Fourteen of these received a large amount of cycloserine in their diet, so that they were constantly under the influence of this drug (group B, table IX). As done previously, all of the rats received, at weekly intervals, 1.0 Gm./Kg. of cycloserine subcutaneously.

The data are summarized in table IX. Both groups A and B received the synthetic diet reinforced with 4 mg./Kg. of pyridoxine hydrochloride for a period of 10 days to insure B<sub>6</sub> saturation. To the diet of group B was added 1.0 per cent of cycloserine, but after six days the animals did not gain weight well and had become lethargic. The concentration of cycloserine was then reduced to 0.75 per cent, at which it was kept for the duration of the experiment.

At the end of the 10 day acclimatization period, all of the rats received subcutaneously 1.0 Gm./Kg. of cycloserine as a provocative dose. On the following day, both groups A and B were put on a B<sub>6</sub> deficient diet by omitting the pyridoxine. In addition, group B was getting 0.75 per cent of cycloserine in the diet, and both groups received subcutaneously, at about weekly intervals, an injection of 1.0 Gm./Kg. for the next 55 days.

TABLE IX  
*Effects of Cycloserine on Normal and Pyridoxine Deficient Rats*

Cycloserine injection	Group	Diet		No.	Days fed	Av. weight, Gm.	Symptoms	
		Pyridoxine, 4 mg./Kg.	Cycloserine, per cent				From B <sub>6</sub> deficiency	From Cycloserine injection*
1	A	+	None	10	0	54.6		None
	B	+	1.0	14	0	53.1		None
2	A	+	None	10	10	87.4		None
	B	+	1.0	14	6	—		
	B	+	0.75	14†	4	66.0		None
3	A	—	None	9‡	21	115.0		None
	B	—	0.75	12	21	85.0		None
4	A	—	None	9	28	141.0		Unsteady
	B	—	0.75	12	28	116.0		Narcosed
5	A	—	None	9	35	139.0	Mod. +	Unsteady
	B	—	0.75	12	35	113.0	Mod. 2+	Narcosed
6	A	—	None	9	40	132.0	Mod. +	Unsteady
	B	—	0.75	12	40	108.0	Mod. 2+	Narcosed
7	A	—	None	8§	50	121.0	Mod. 2+	Unsteady
	B	—	0.75	12	50	102.0	Mod. 3+	Narcosed
8	A	—	None	8	60	112.0	Marked +	Unsteady
	B	—	0.75	12	60	93.0	Marked 3+	Narcosed

\* Dose of 1.0 Gm./Kg., given subcutaneously.

† Sacrificed for tissue study (2 animals).

‡ One animal died at 15 days.

§ One animal died at 42 days.

There was a slower rate of development of B<sub>6</sub> deficiency symptoms (table IX), compared to the animals of the first series (table VIII), probably because of better preliminary saturation with this vitamin. The lesser rate of weight loss and lower mortality allowed a more detailed observation of symptoms due to cycloserine feeding.

The preliminary addition of 1.0 per cent of cycloserine to the diet slowed the rate of gain, so that the two groups were unequal in weight at the time of removal of B<sub>6</sub> from the diet. As the experiment progressed, there were three areas of difference between the cycloserine-fed rats and the controls: (1) The gain in weight was slower, reached a smaller peak, and declined more rapidly; (2) the signs of B<sub>6</sub> deficiency appeared about at the same time in the two groups. However, these were definitely more severe in the rats on the cycloserine-fortified diet; and, (3) the animals' reactions to the weekly subcutaneous injection of 1.0 Gm./Kg. of cycloserine was both quantitatively and qualitatively different in the two groups.

Those animals not receiving the drug in the diet (group A) reacted to the injection by unsteadiness, progressing with time to jerky movements of the hind legs, lasting as long as two hours in a few of the animals. The animals receiving the 0.75 per cent cycloserine in the diet (group B) showed a greater degree of unsteadiness and in greater number following the injection. Moreover, these animals became deeply depressed and narcosed, except that they reacted to stimulation by jerky movements and changes in position or kicking with their hind legs.

Between the injections of cycloserine, all rats showed hyperreflexia to touch or sudden sounds, which was more marked in the cycloserine-fed animals. Neither group reacted inordinately to a bright light stimulus. There were no frank convulsions. By the sixty-fifth day the animals of both groups had lost weight and had advanced signs of B<sub>6</sub> deficiency. They were sacrificed for tissue study. Only the spleen was smaller than normal, probably the result of lack of proper growth.

#### SUMMARY

Clinical evidence is presented on 27 tuberculous patients treated with large doses of cycloserine, which shows that pyridoxine reduces the toxicity and prevents the convulsions that may occur with cycloserine. This new regimen allows the successful treatment of tuberculosis due to drug-resistant bacilli and permits the rehabilitation of a large proportion of the patients who are otherwise doomed to invalidism and early death.

Experimental evidence in rats failed to connect the cycloserine toxicity with vitamin B<sub>6</sub> depletion. It is concluded that the salutary effects of pyridoxine in lessening the cycloserine toxicity are pharmacological, rather than vitaminic, in nature.

#### ACKNOWLEDGMENTS

The cycloserine and pyridoxine were generously supplied by Eli Lilly & Co. The synthetic diets and rats used in this study were obtained from the Animal Care Department, Columbia College of Physicians and Surgeons, through the courtesy of Dr. Slanetz.

#### BIBLIOGRAPHY

1. NAIR, K. G. S.: Transactions of the 16th Veterans Administration-Armed Forces Conference on the Chemotherapy of Tuberculosis, Feb., 1957, p. 56.

2. EPSTEIN, I. G.; NAIR, K. G. S., AND BOYD, L. J.: Cycloserine-pyridoxine combinations in the control of cycloserine toxicity during treatment of chronic, resistant pulmonary tuberculosis. *In: Transactions of the 17th Veterans Administration-Armed Forces Conference on the Chemotherapy of Tuberculosis*, Feb., 1958, p. 34.
3. NAIR, K. G. S.; EPSTEIN, I. G.; MULINOS, M. G., AND HABER, A.: Reduction of cycloserine neurotoxicity by pyridoxine. Presented at American Society of Pharmacology and Experimental Therapy, Ann Arbor, Mich., Aug. 25-28, 1958.
4. MURRAY, F. J.: A pilot study of cycloserine toxicity, *Am. Rev. Tuberc.* 74:196, 1956.
5. STOREY, P. B.; DUVAL, R. C., AND MCLEAN, R. L.: The convulsive effect of cycloserine, *AM & CT* 3:258, 1956.
6. STOREY, P. B., AND MCLEAN, R. L.: Some considerations of cycloserine toxicity, *Am. Rev. Tuberc.* 75:514, 1957.
7. EPSTEIN, I. G.; NAIR, K. G. S., AND BOYD, L. J.: The treatment of human tuberculosis with cycloserine. A year's progress. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, pp. 141-147.
8. ANON.: New and nonofficial drugs, cycloserine, *J.A.M.A.* 163:645, 1957.
9. BARCLAY, W. R.; PYLE, M.; POIRIER, P., AND PFUETZE, K. H.: High doses of cycloserine plus phenobarbital and Dilantin for the treatment of pulmonary tuberculosis, *Chemo. of Tuberc.* 16:57, 1957.
10. NAIR, K. G. S.; EPSTEIN, I. G.; BARON, H., AND MULINOS, M. G.: Absorption, distribution, and excretion of cycloserine in man. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, pp. 136-140.
11. OSOL, A., AND FARRAR, JR., G. E.: *U. S. Dispensatory*, Ed. 25, Philadelphia, J. B. Lippincott Co., 1955, p. 726.
12. REILLY, R. H.; KILLAM, K. F.; JENNEY, E. H.; MARSHALL, W. H.; TAUSIG, T.; APTER, N. S., AND PFEIFFER, C.: Convulsant effects of isoniazid, *J.A.M.A.* 152:1317, 1953.
13. D'ESOP, N.: Chemotherapy of tuberculosis in man, *J.A.M.A.* 154:52, 1954.
14. MOLONY, C. J., AND PARMELEE, A. H.: Convulsions in young infants as a result of pyridoxine (vitamin B<sub>6</sub>) deficiency, *J.A.M.A.* 154:405, 1954.
15. COURSIGN, D. B.: Convulsive seizures in infants with pyridoxine deficient diet, *J.A.M.A.* 154:406, 1954.
16. ANON.: The pyridoxine requirements of infants, *Nutrition Rev.* 16:10, 1958.
17. EPSTEIN, I. G.; NAIR, K. G. S., AND BOYD, L. J.: The treatment of human pulmonary tuberculosis with cycloserine: Progress report, *Dis. of Chest* 29:241, 1956.
18. OLANSKY, S.: The Herxheimer reactions of relatively small doses of penicillin, *J. Ven. Dis. Inform.* 28:26, 1947.
19. FARMER, T. W.: Jarisch-Herxheimer reaction in early syphilis treated with crystalline penicillin-G, *J.A.M.A.* 138:480, 1948.
20. STOREY, P. B.: Results of original course of cycloserine chemotherapy: Pilot studies IX (CS), XII (CS-INH) and XIII (CS-PZA-INH): Data from cooperative study, *Transactions of the 16th Veterans Administration-Armed Forces Conference on the Chemotherapy of Tuberculosis*, Feb., 1957, p. 44.
21. KAUNITZ, H.; SLANETZ, C. A., AND ATKINSON, W. B.: Estrogen response and pigmentation of the uterus in vitamin E-deficient rat, *Proc. Soc. Exper. Biol. & Med.* 70:302, 1949.
22. BIEHL, J. P., AND VILTER, R. W.: Effect of isoniazid on vitamin B<sub>6</sub> metabolism: its possible significance in producing isoniazid neuritis, *Proc. Soc. Exper. Biol. & Med.* 85:389, 1954.
23. VILTER, R. W.; BIEHL, J. P.; MUELLER, J. F., AND FRIEDMAN, B. I.: Some abnormalities of vitamin B<sub>6</sub> metabolism in human beings, *Federation Proc.* 13:776, 1954.
24. NAIR, K. G. S.: Cycloserine combination therapy discussion, *Transactions of the 16th Veterans Administration-Armed Forces Conference on the Chemotherapy of Tuberculosis*, Feb., 1957, p. 53.

# Effect of a New Hormone, Triamcinolone, When Combined with the Standard Treatment of Tuberculosis

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After the discovery of cortisone and hydrocortisone, various investigators devoted themselves to looking for new compounds which, while retaining the same therapeutic properties, would be free of the inconveniences usually associated with the use of such hormones. Efforts were thus made to obtain a substance with greater glucocorticoid action, more anti-inflammatory action, and less mineralcorticoid activity. A decisive forward step was the advent of prednisone and prednisolone, already tested on a large scale with much success, and to whose effect on certain forms of tuberculosis we have referred elsewhere.<sup>1, 2</sup>

Bernstein et al<sup>3</sup> recently synthesized a new derivative of cortisone, naming it triamcinolone.\* This is a hydrofluoric derivative, the complete formula for which is 9 $\alpha$ -fluoro-16 $\alpha$ -hydroxy- $\Delta^1$ -hydrocortisone. Its structural formula is shown in figure 1.

The glucocorticoid activity of this drug, in terms of the amount of glycogen that is stored in adrenalectomized rats, is 15 to 36 times that of hydrocortisone acetate when both are used subcutaneously. Triamcinolone is wholly devoid of mineralcorticoid action, i.e., it does not cause retention of water, chlorine, or sodium, nor does it increase the excretion of potassium. On the contrary, it is a surprising fact that this drug, when administered in subcutaneous injections to adult dogs, possesses diuretic action, with increased sodium excretion and no action on potassium. In rats it does not originate hypertension. It is more active than hydrocortisone or prednisone for preserving life in adrenalectomized rats. It has neither androgenic nor estrogenic activity.

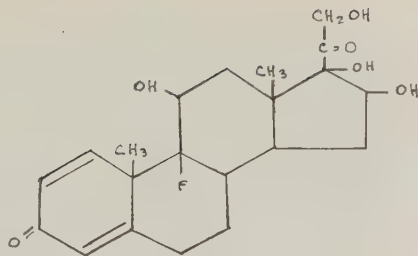
This new cortisone derivative has already been used clinically in human beings. Its indications are the same as those for prednisone, the therapeutic effect of both being essentially the same. But triamcinolone is more effective as an anti-inflammatory substance, and smaller dosages are therefore needed to obtain similar results. Feinberg et al<sup>4</sup> estimated that the dosage must be half that of prednisone; he has also noticed that triamcinolone does not always increase appetite and at times even decreases it. Its diuretic action may be very favorable in some patients, such as those with heart failure and edema, but in others it may be undesirable. Side effects appear as when prednisone is administered; caution must be exercised against hyperglycemia, muscular cramps, gastric disturbances, dizziness, headaches.

The use of triamcinolone in 26 patients with severe dermatoses gave good results.<sup>5</sup> The anti-inflammatory and antipruriginous actions of this drug are superior to those of prednisone, and almost no side effects have been noticed.

Shelley et al<sup>13</sup> likewise reported very encouraging results with triamcinolone in several cases of psoriasis and other severe dermatoses. Naturally enough, they in-

\* The trade name of Lederle Laboratories Division, American Cyanamid Co., for triamcinolone is Aristocort. The triamcinolone used in these experiments was supplied by Lederle Laboratories.

FIG. 1. This is the structural formula for triamcinolone.



sisted that they do not believe the drug is truly curative, but only palliative, since symptoms reappear on discontinuance of the drug. They consider that the useful daily dosage is half that of either prednisone or prednisolone.

Hellman et al<sup>6</sup> have used this new drug on patients with rheumatoid arthritis, and good results have been obtained with no impairment of metabolism during the treatment.

Patients with asthma have also been seen to improve with triamcinolone. Sherwood and Cooke<sup>7</sup> treated 16 such patients for intervals of 4 to 11 weeks and most of them exhibited a remarkable improvement. The average dosage used by these investigators was 6 mg./day.

Other types of diseases have also proved to benefit decisively with this hormone: disseminated lupus erythematosus, nephrotic syndrome, psoriasis, leukemia, disturbances of the lymphatic system, and many others.

The daily dosage to be used in each case must be determined by the practicing physician. As a general guide, it may be remembered that the dosage must represent half the respective dosage of prednisone. On initiating treatment with triamcinolone, it should be borne in mind that we are handling a new drug with which there is but little clinical experience and care must be exercised. Dosage must be small at the beginning, to be increased as allowed by tolerance.

#### HORMONE THERAPY IN TUBERCULOSIS

The use of cortisone and its derivatives in various forms of tuberculosis, both experimental and in the human clinic, has been the subject of several experiences and communications lately.<sup>8,9</sup> The altered evolution in guinea pigs with induced tuberculosis after incorporation of these substances with drugs used for therapeutic or prophylactic purposes has been extensively studied; it is also known that cortisone has little effect on reaction to bacille Calmette Guérin vaccine.<sup>14</sup> In man experiences have already accumulated, even though no full agreement has been reached as yet on the drug's use and indications.<sup>10,11</sup> An exhaustive, up-to-date survey on this subject has just been presented by Sayé<sup>12</sup> in his recent book on the management of tuberculosis. On two occasions we have reported<sup>1,2</sup> on the advantageous adjunct of these hormones to the classical antituberculosis treatment, as well as on what we hold to be the precise indications for this, especially the requirements to be met when administering corticosteroids to a tuberculous patient. For the sake of brevity, the reader is referred to these communications.

As soon as triamcinolone, the last derivative of cortisone, appeared, we immediately began using it for tuberculous patients on the basis of experience gained with prednisone and changes suggested in connection with the use of this new drug. As an essential indication we have linked hormone therapy to severe cases, in patients whose general condition was already impaired and who had not had any specific treatment, or in those who had had treatment and showed laboratory evidence that

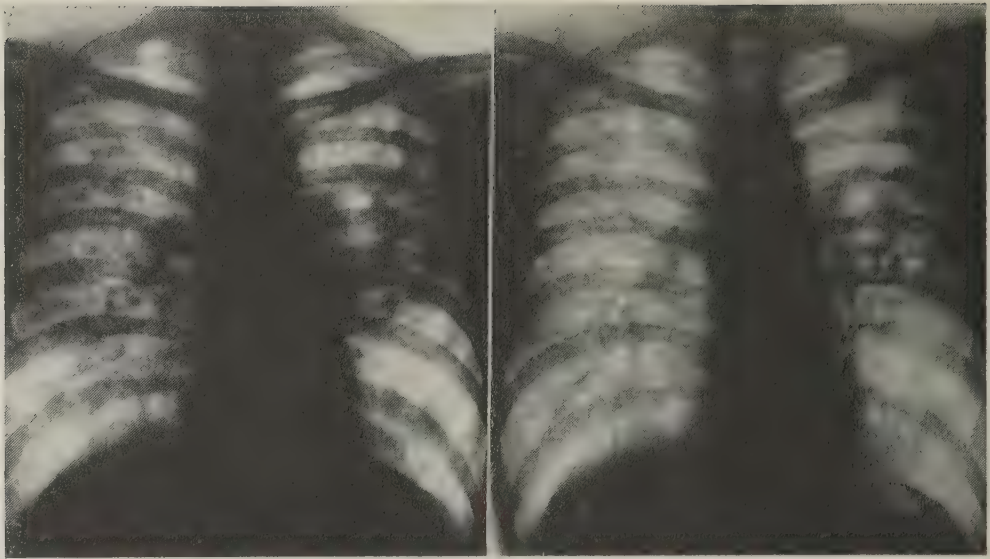


FIG. 2. (Left) Roentgenograph of lungs of patient R. M. shows extensive lesions.

FIG. 3. (Right) Roentgenograph of lungs at the end of one month indicates improvement.

the Koch bacilli were still sensitive to isoniazid or streptomycin. We consider it advisable to stress this last requirement when adding hormones to the medication given to a tuberculous patient, in view of the fact that current literature tends to suggest that this requirement is not always borne in mind. It would be unwise to overlook the great risk involved in subjecting to cortisone therapy a patient whose germs are still resistant to isoniazid, streptomycin, or both.

Besides those patients who are first seen with greatly advanced tuberculosis, we have used triamcinolone for those tuberculous patients who, after having been treated for several weeks or months, do not show any improvement either clinically or on roentgen-ray examination. In all of them, only a few days after treatment is begun, an improvement is noted, in terms of well-being, lowering of temperature, and better appetite. Roentgenograms do not always show dramatic changes.

When we used prednisone, the daily dosage for adult patients was almost 10 mg. With triamcinolone the dosage is 4 mg./day. The duration of treatment is short, from four to six weeks.

We believe that these forms of tuberculosis benefit greatly by this combined medication, and, therefore, provided the prescribed requirements are complied with, all such patients should be treated in this manner. On the other hand, the lack of any kind of reaction attributable to the hormone eliminates any fear the physician might entertain.

The following case is an example of severe tuberculosis.

R. M., a 38 year old white Argentine man, began to have respiratory symptoms in August, 1957, but kept working until May 2, 1958, when he was admitted on Ward 5 of Muñiz Hospital. He had had no previous treatment. On admission to the hospital he was emaciated, feverish, and a roentgen-ray examination showed extensive lesions in both lungs (fig. 2). The sputum contained Koch bacilli, which proved sensitive to both streptomycin and isoniazid. Treatment was commenced with daily administrations of isoniazid and para-aminosalicylic acid and 4 mg. of triamcinolone. At the end of one month his weight had increased by 6 Kg., his general appearance had improved visibly, and roentgen-ray examination showed a favorable course (fig. 3). Triamcinolone was then suspended, while continuing with anti-tuberculosis drugs.

Some tuberculous patients also have bronchial asthma. Although the asthma

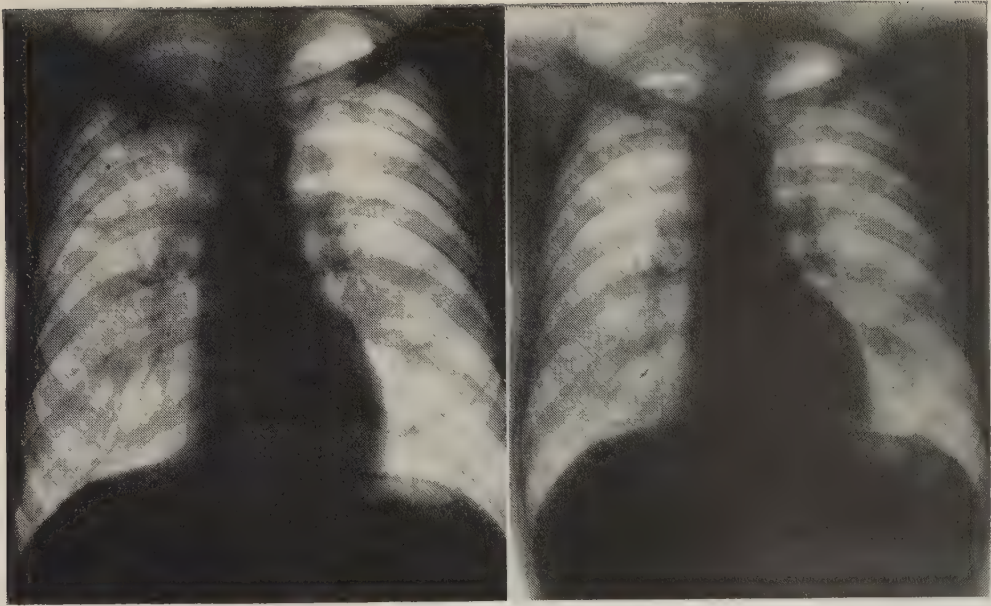


FIG. 4. (*Left*) Roentgenograph of chest of patient J. B. shows lesions in the right lung.  
 FIG. 5. (*Right*) Improvement in lesions at the end of three months' treatment is shown.

improves in some patients after starting on a treatment for tuberculosis, this is not always the case. The beneficial effects of cortisone on asthmatic patients being undeniable, the fact that this drug can be administered in the presence of tuberculosis is a further advantage from the viewpoint of the patient's well-being.

We have treated patients with pulmonary tuberculosis and asthma by combining triamcinolone with classical antituberculosis medication. The results have been excellent in both conditions, and even in cases where tuberculosis is not severe, it is desirable that during its healing period the patient should have no attacks of asthma that may bear unfavorably on the development of bacillary lesions. In such cases, the duration of treatment with the hormone is longer and maximum benefit with minimal dosage is strived for.

J. B., a 47 year old white Argentine man, entered Ward 5 of Muñiz Hospital on Nov. 19, 1957. He had had bronchial asthma for four years. Tuberculosis of the lungs began in April, 1957, but he did not apply for admission at the hospital until November. Roentgen-ray examination of the chest (fig. 4) showed lesions in the right lung. Treatment was begun with streptomycin and isoniazid. Asthmatic spells were frequent and it was hard to control them with routine medication; in view of this, on May 6, triamcinolone was combined in 2 mg. daily dosage, with the asthmatic disturbances immediately disappearing. The patient has been on this combined therapy three months and his pulmonary lesions show a decisive improvement. There is no intolerance whatsoever to triamcinolone therapy (fig. 5).

An interesting case of tuberculosis combined with fully active malaria showed undeniable benefit on combination of triamcinolone and antituberculosis drugs.

W. D., a 47 year old white Argentine man, contracted malaria in childhood and was treated, but never completely. He had also had syphilis, which was properly treated and cured. Pulmonary tuberculosis began in 1949 with left cavitary lesions. He was treated with streptomycin, isoniazid, and para-aminosalicylic acid on various occasions, but always incompletely, his disease remaining active. He was admitted to the Buenos Aires Sanatorium on April 29, 1958, with severe respiratory symptoms; he was extremely dyspneic, with cough and abundant purulent expectoration. He had high fever, with large fluctuation and classical afternoon chills, which he had had for some years. He was treated after admission with streptomycin, isoniazid, and para-aminosalicylic acid. The malarial manifestations did not yield. On May

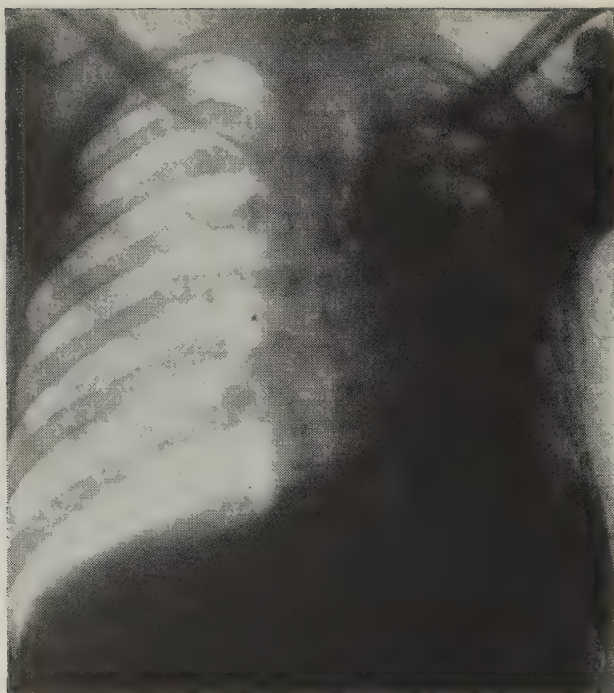


FIG. 6. Patient W. D. Improvement in lesions of lung are shown at the end of three months of treatment.

19, 1958, a thick-drop blood examination showed the presence of *Plasmodium vivax*. On May 5 triamcinolone was added in 4 mg. daily dosage and both fever and chills disappeared immediately. This treatment was continued for three months, improvement being noticed both clinically and on roentgen-ray examination in the lesions of the lung during this period (fig. 6).

Another indication for combining triamcinolone with the specific treatment of tuberculosis is when the patient shows severe manifestations of intolerance to some of the medications. Of the three main drugs, streptomycin, para-aminosalicylic acid, and isoniazid, the first two more frequently originate such disturbances as to make

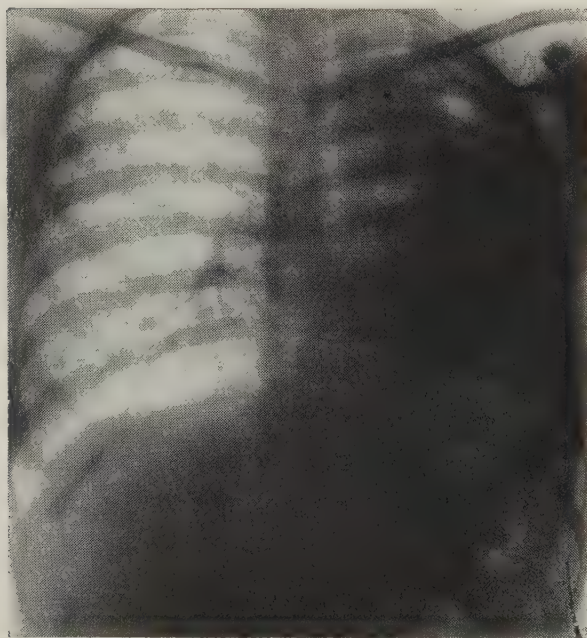


FIG. 7. Patient J. deG. Roentgenographic examination shows destruction of the left lung.

their discontinuance mandatory. Intolerance to para-aminosalicylic acid is more significant when the patient has Koch bacilli that have already become resistant to either streptomycin or isoniazid. In such cases, administration of para-aminosalicylic acid combined with the other useful medication is most advisable. If this drug brings about signs of intolerance of an allergic or other type, the administration of triamcinolone removes such symptoms and allows the patient to benefit through combined treatment. It has been possible for Houghton and Davies<sup>14</sup> to treat 8 patients who exhibited hypersensitivity to streptomycin or para-aminosalicylic acid; on addition of cortisone, the disturbances disappeared and the patients could tolerate these drugs. But it is also striking that in 6 of these patients, the signs of intolerance disappeared on discontinuance of hormone therapy after several weeks.

J. de G., a 23 year old white Argentine woman, was admitted to the Koch Pavilion on Dec. 24, 1957. Her illness had apparently begun in March, 1957, after her second childbirth. Roentgen-ray examination of the chest and laboratory assays showed the presence of tuberculosis of the lungs with cavities on the left side. She was treated on several occasions with streptomycin and isoniazid, pneumoperitoneum and left pneumothorax, receiving a total of 480 Gm. of streptomycin and 89 Gm. of isoniazid. On admission, her sputum showed Koch bacilli, and roentgen-ray examination showed destruction in the left lung (fig. 7). Also, she was pregnant, with expected delivery in March, 1958, at which date birth of her fifth child was normal. Her general condition was very feeble. An antibiogram was made and Koch bacilli proved to be sensitive to streptomycin and resistant to isoniazid (1 gamma). Treatment was then started with streptomycin and para-aminosalicylic acid, but a severe urticaria-like skin reaction appeared early, with fever and general malaise. On two occasions this drug was discontinued and reinstated, urticaria disappearing and reappearing coincidentally. It was then decided to add triamcinolone in 4 mg. daily dosage, and from that time, tolerance to para-aminosalicylic acid became perfect. The patient has already had three months' treatment with streptomycin, para-aminosalicylic acid, and triamcinolone and she is improving gradually. The future plan includes a surgical operation, which will be performed once the patient's condition is stabilized.

#### SUMMARY

Corticosteroids can be very efficient, when used in conjunction with the specific treatment, in certain forms of tuberculosis. The present writers have adopted this therapeutic combination since 1955. In the beginning, cortisone was used, then prednisone, and finally triamcinolone. At present, the latter offers the greatest advantages. The following conditions are essential when employing this hormone for the treatment of tuberculosis: that the organisms be sensitive to the drugs used, that isoniazid be included in the treatment, and that the blood glucose be at a normal level.

The best indications are: very severe tuberculosis (including meningitis), tuberculosis associated with another disease where an improvement is possible through corticotherapy (asthma, arthritis), hypersensitivity to some antituberculosis medication. This report presents several examples of such conditions.

After three years' experience, the present writers recommend the addition of hormone therapy for tuberculosis and consider that it greatly benefits the condition of the patient.

#### BIBLIOGRAPHY

1. PILHEU, J. A., AND IANNELLO, J. A.: Cortisona en la tuberculosis pulmonar, *Prensa méd. Argent.* 51:3747, 1956.
2. PILHEU, J. A., AND IANNELLO, J. A.: Los corticosteroides en la tuberculosis humana, *Prensa méd. Argent.* 53:294, 1958.

3. BERNSTEIN, S.; LENHARD, R. H.; ALLEN, W. S.; HELLER, M.; LITTELL, R.; STOLLAR, S. M.; FELDMAN, S. I., AND BLANK, R. H.: 16-Hydroxylated steroids. IV. Synthesis of 16 $\alpha$ -hydroxy derivatives of 9 $\alpha$ -halosteroids, Communications to the Editor, J. Am. Chem. Soc. 78:5693, 1956.
4. FEINBERG, S.; FEINBERG, A. R., AND FISHERMAN, E. W.: Triamcinolone (Aristocort), new corticosteroid hormone: its use in treatment of allergic disease, J.A.M.A. 167:58, 1958.
5. REIN, C. R.; FLEISCHMAJER, R., AND ROSENTHAL, A. L.: Use of a new corticosteroid (Aristocort diacetate) in dermatology, J.A.M.A. 165:1821, 1957.
6. HELLMAN, L.; ZUMOFF, B.; SCHWARTZ, M.; GALLAGHER, T.; BERNTSEN, C., AND FREYBERG, R.: Antirheumatic and metabolic effects of new synthetic steroid, read before the interim meeting, American Rheumatic Association, National Institutes of Health, Bethesda, Md., Nov. 30, 1956.
7. SHERWOOD, H., AND COOKE, R. A.: Preliminary evaluation of new corticosteroid in allergic diseases, J. Allergy 28:97, 1957.
8. TSUJI, S.; HEKI, S.; ITO, K.; OSHIMA, S., AND TAKEAKA, A.: The possible role of humoral factors in the enhanced growth of tubercle bacilli in rabbits given cortisone, Am. Rev. Tuberc. 77:529, 1958.
9. LURIE, M.: Ann. New York Acad. Sc. 56:779, 1953.
10. HOUGHTON, L. E.: Combined corticotrophin therapy and chemotherapy in pulmonary tuberculosis, with special reference to hypersensitive reactions, Lancet 1:594, 1954.
11. JEUNE, M.; CHARRAT, A., AND BIOT, J.: Pédiatrie 13:459, 1958.
12. SAYÉ, L.: Tratamiento y Profilaxis de la Tuberculosis, Barcelona, Salvat, 1958.
13. SHELLEY, W. B.; HARUN, J. S., AND PILLSBURG, D. M.: The treatment of psoriasis and other dermatoses with triamcinolone (Aristocort), J.A.M.A. 167:959, 1958.
14. HOUGHTON, L. E., AND DAVIES, D.: Effect of cortisone in BCG vaccination in guinea pigs, Tubercle 35:2, 1954.

# Isolation of a New Antibiotic Related to Aburamycin

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A new antimicrobial agent, M5-18903, has been isolated from a strain of *Streptomyces*. This antibiotic appears to be the optical antipode of aburamycin, isolated by Nishimura et al.<sup>1</sup> In this paper, studies on the production, isolation, and characterization of M5-18903 are presented. The properties of this antibiotic are compared with those of aburamycin.

## FERMENTATION

M5-18903 is produced by a strain of *Streptomyces* that was isolated from a soil sample from the Marshall Islands. Preliminary taxonomic studies\* have demonstrated that this organism can be readily differentiated from the organism that produces aburamycin. The complete report on the taxonomic studies will be submitted elsewhere.

The organism was maintained on agar slants that contained asparagin, beef extract, and peptone. The spore inoculum was transferred to 100 ml. of vegetative media (table I) in a 500 ml. flask, and the organism was incubated for 48 hours at 30 C. Good aeration was attained by shaking on a reciprocal shaker. A 5 per cent inoculum was transferred to fermentation media (table I) in shaken flasks or stirred fermentation tanks. The antibiotic potency of the broths was checked by a four hour turbidimetric assay with *Micrococcus pyogenes* var. *aureus*. Maximum yields were obtained when the fermentation was carried out at 30 C. for 72 to 96 hours. Paper chromatographic procedures showed that the activity produced did not correspond to any of the known antibiotics. Subsequently, an authentic sample of aburamycin was obtained. The behavior of aburamycin in a number of paper chromatographic procedures was identical with that of M5-18903.

## PURIFICATION

A procedure for the preparation of crystalline M5-18903 is as follows. The filtered broth at pH 7.2 to 7.5 was extracted with one half volume of chloroform. A crude preparation was obtained by precipitating the chloroform extract with several volumes of petroleum ether. The precipitate, ranging in color from yellow-green to yellow-brown, was washed with petroleum ether and air-dried. The dried powder was then dissolved in a small volume of chloroform and passed over an alumina column (acid-washed) prepared with chloroform. The column was developed with chloroform and the antibiotic was eluted with 5 per cent ethanol in chloroform. The active eluates were combined, concentrated under reduced pressure to a low volume, and precipitated by the addition of several volumes of petroleum ether. The active precipitate was washed with petroleum ether and air dried to yield a bright yellow powder, which was forty- to fiftyfold purified from the crude broth. Crystallization was effected from a concentrated chloroform solution to which petroleum ether was added.

The procedure of Nishimura et al.<sup>1</sup> could be used as well to isolate the anti-

\* These data were obtained by Mr. C. E. Higgins.

TABLE I  
Media Composition

Vegetative medium	Gm./liter	Fermentation medium	Gm./liter
Cerelose	15	Cerelose	40
Soy bean meal	15	Soy bean meal (expeller type)	20
Sodium chloride	5	B-Y 500	5
Calcium carbonate	2	Sodium chloride	5
Corn steep (8 ml.)		Casein	5
pH adjusted to 6.0 before autoclaving		Defoaming oil (Dow P-2000), 0.2 ml.	
		pH adjusted to 6.0 before autoclaving	

biotic. This procedure is as follows: The fermentation broth, after filtration at pH 9.5, is extracted into an equal volume of ethyl acetate at pH 2.0. The ethyl acetate extract is washed with water and the wash is discarded. The antibiotic is then transferred into water at pH 9.0. The extraction and counterextraction are repeated two times with decreasing volumes of chloroform. The final chloroform extract is concentrated in vacuo to a low volume and chromatographed on an alumina (acid-washed) column. The column is developed and eluted with 80 per cent methanol. The active (yellow-colored fractions are pooled, concentrated to a low volume, and held at 10 C. to effect crystallization. However, crystals of M5-18903 have not been obtained by this procedure.

#### CHARACTERIZATION AND CHEMICAL PROPERTIES

M5-18903 is a weakly acidic antibiotic, soluble in chloroform, acetone, pyridine, ethyl acetate, dimethyl formamide, ethanol, and methanol, but insoluble in water and petroleum ether. Electrometric titration shows one ionizable group with  $pK_a$  of 7.1 in 66 per cent dimethyl formamide. The apparent molecular weight is 1295.

The compound was hydrogenated in alcohol using a platinum catalyst. Two moles of hydrogen were taken up in one hour and no further reduction was noted. The compound was acetylated using pyridine and acetic anhydride at room temperature overnight to yield a crystalline product that contained 22.94 per cent acetyl by microanalysis. The reduced material retained practically the same activity

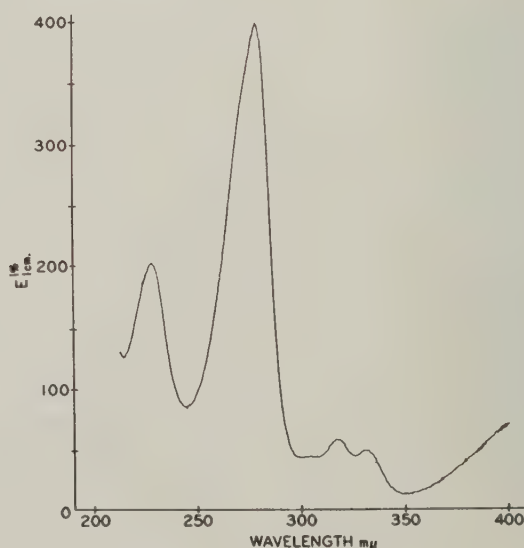


FIG. 1. The ultraviolet spectrum of M5-18903 is shown.

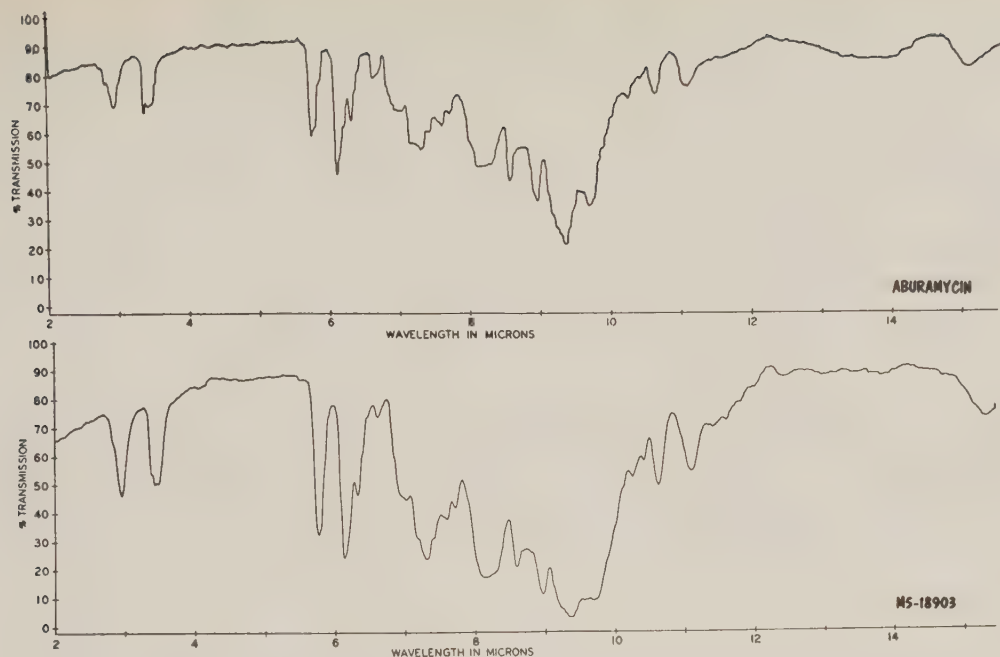


FIG. 2. The infrared spectra of aburamycin and of M5-18903 in chloroform are compared.

as the parent compound, but acetylation of the antibiotic completely destroyed the activity. The acid melts at 169 to 171 C. and the acetyl derivative melts at 205 to 207 C.

The ultraviolet spectrum (fig. 1) is characterized by maxima in alcohol at 227  $\mu$ , 228  $\mu$ , and 400  $\mu$  and weak maxima at 304  $\mu$ , 317  $\mu$ , and 330  $\mu$ .  $E_{1\text{cm.}}^{1\%}$  (227  $\mu$ )  $200 \pm 5$ ;  $E_{1\text{cm.}}^{1\%}$  (278  $\mu$ )  $400 \pm 10$ ;  $E_{1\text{cm.}}^{1\%}$  (317  $\mu$ )  $60 \pm 2$ . The ultraviolet spectrum of M5-18903 is quite similar to that of aureolic acid, isolated by Philip and Schenck.<sup>2</sup> The ultraviolet spectra of the hydrogenated products obtained from these two antibiotics are similar in both acid and alkaline solution. The infrared spectrum in chloroform is shown in figure 2 and is compared with that of aburamycin. Analytical data are as follows: Found: C, 56.32, H, 7.44, O, 36.24; no nitrogen, sulfur, or halogen. The specific rotation of M5-18903 is  $[\alpha]_D^{25} = -29^\circ$  ( $c = 0.5$  per cent in methanol). The reported rotation of aburamycin is  $[\alpha]_D^{20} = +24.56^\circ$  ( $c = 1$  per cent in methanol).<sup>1</sup> In our hands, a small sample of aburamycin gave a specific rotation  $[\alpha]_D^{25} = +28^\circ \pm 5^\circ$ . These two specific rotation determinations of M5-18903 and aburamycin are of equal values, within experimental

TABLE II  
In Vitro Antimicrobial Spectrum of M5-18903

Test organism	Inhibitory concentration, $\mu\text{g.}/\text{ml.}$ , 24-48 hr.			
	18903	free acid	18903	hydrogenated
<i>Micrococcus pyogenes</i> var. <i>aureus</i>	0.05	0.1	0.2	0.2
<i>Micrococcus pyogenes</i> var. <i>albus</i>	0.2	0.2	0.4	0.4
<i>Bacillus subtilis</i>	0.05	0.1	0.1	0.2
<i>Mycobacterium phlei</i>	0.78	0.78	1.56	3.13
<i>Mycobacterium tuberculosis</i> (607)	—	0.78	—	0.78
<i>Mycobacterium avium</i>	0.78	3.13	1.56	12.5
<i>Brucella bronchiseptica</i>	100	100	100	100
<i>Corynebacterium sepeodonicum</i>	0.2	(72 hr.)	0.4	(72 hr.)

TABLE III  
Comparison of M5-18903 and Aburamycin

	M5-18903	Aburamycin
Carbon	56.32%	55.57%
Hydrogen	7.44%	7.54%
Oxygen	36.24% (difference)	36.89%
Melting point	169–171 C.	163–165 C.
Rotation	$[\alpha]_D^{25} = -29^\circ$ (c = 0.5% in methanol)	$[\alpha]_D^{20} = +24.56$ (c = 1% in methanol)
Ultraviolet spectrum	Maxima at 227 $\mu$ , 278 $\mu$ , and 400 $\mu$ ; weak maxima at 304 $\mu$ , 317 $\mu$ , and 330 $\mu$	Maxima at 230 $\mu$ , 276 $\mu$ , and 410 $\mu$ ; weak maxima at 320 $\mu$ , and 330 $\mu$ , and 350 $\mu$

error, but are of opposite sign. To our knowledge this phenomenon of two active, naturally isolated isomers of an antibiotic has never been reported. Paper chromatography analysis indicates the two antibiotics to be indistinguishable, as does the analysis of the infrared and ultraviolet spectra. All of these data, together with the microanalysis comparison, tend to indicate that the two antibiotics, M5-18903 and aburamycin, are optical antipodes.

#### BIOLOGICAL ACTIVITY

The in vitro antimicrobial spectrum of M5-18903, as determined by the usual agar dilution tests (Penassay seed agar), is shown in table II. The antibiotic is effective against the gram-positive organisms and the mycobacteria, but ineffective against gram-negative organisms. Table II also compares the relative activities of the free acid and the hydrogenated materials. A comparison of some of the analytical and physical data of M5-18903 and aburamycin is shown in table III.

Acute intravenous toxicity tests in mice indicate an LD<sub>50</sub> dose of 2 mg./Kg., and chronic studies show the antibiotic to be accumulated and unabsorbed at the site of injection. Single doses of 80 mg./Kg. orally were tolerated by mice, and amounts of up to 488 mg./Kg. in a diet were consumed by mice over a five day period with no toxic symptoms noted.

M5-18903 showed some effectiveness against *Entamoeba histolytica* in vivo and also reduced the worm burden in *Syphacia obvelata*-infected mice. When tested against mice infected with sarcoma 180 tumors, M5-18903 was ineffective. When administered to leukemia P-1534-infected mice at levels of 1.5 to 3.0  $\mu$ g./Kg., the antibiotic showed 25 to 32 per cent protection. Higher dosage levels proved to be toxic and killed the test animals within a 24 hour period.

#### SUMMARY

The chemical and biological properties of M5-18903, a new antibiotic from a *Streptomyces* species, have been described. The antibiotic appears to be the optical antipode of aburamycin.<sup>1</sup> M5-18903 is active in vitro against gram-positive organisms and mycobacteria but has little therapeutic effect at subtoxic dosages. It does, however, exhibit some effectiveness against mouse leukemia P-1534.

#### BIBLIOGRAPHY

1. NISHIMURA, H.; KEMURA, T.; TAWARA, K.; SASAKI, K.; NAKAJIMA, K.; SHIMAOKA, N.; OKAMOTO, S.; SHIMOHIRA, M., AND ISONO, J.: Aburamycin, a new antibiotic, *J. Antib.* 10: 205–212, 1957.
2. PHILIP, J. E., AND SCHENCK, J. R.: Aureolic acid, a new antibiotic. II. Isolation and properties, *Antib. & Chemo.* 3:1218, 1953.

# Production of Oncostatic Principles In Vivo and In Vitro by Species of the Genus *Calvatia*

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In the following the discovery of tumor-inhibiting properties of preparations from fresh and deep-frozen sporophores of several species of *Calvatia* is reported. Also, an account is given of the successful production of mycelium of *Calvatia gigantea* (Pers.) Lloyd in surface as well as in submerged cultures. It was possible to produce the tumor-inhibiting activity in shake cultures at will.

In an earlier paper<sup>1</sup> we presented evidence of the occurrence of tumor inhibitors in other holobasidiomycetes. When this study was continued, it was found that the puffballs with their sometimes extremely large sporophores were a very suitable raw material. Oncostatic activity of this material was first established in 1954. Subsequently, the initial finding was confirmed with large numbers of puffballs, some of which might have belonged to species other than *C. gigantea*, which not only is the most abundant one but also the most suitable because their sporophores are by far the largest. Many of the immature specimens could not be identified because maturity is a requirement for identification; only at maturity the spore characteristics as well as others essential for this purpose can be determined. At present specimens of the species *C. gigantea*, *C. Bovista*, *C. craniformis*, and *C. cyathiformis* are known to produce tumor inhibitors. These were among more than 300 specimens tested between 1954 and 1958. In most of them activity was found. Some, however, exhibited much higher activity than others. In general, it was found that the immature specimens are more reliable sources of the active principle than the mature ones, since they are, as a rule, devoid of toxic substances that develop quite prominently in mature specimens and interfere with the determination of activity in animal tests when crude extracts are used. Although it is possible to separate the toxic substances from the active ones, this is not feasible in mass testing.

The nature of the active principle is still unknown. It is possible that several inhibitors are present, all of which seem to have a retarding effect on the growth of Crocker mouse sarcoma 180.

## EXPERIMENTAL

For preparation of active extracts either fresh puffballs were used, or material was rapidly frozen immediately after collection at  $-10$  to  $-20$  C. and extracted subsequently. Aqueous extracts, when injected into female Swiss albino mice into which sections of Crocker mouse sarcoma 180 had been implanted 24 hours earlier,<sup>2</sup> inhibited tumor growth, as calculated from the average diameter of the tumor, by 25

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TABLE I  
*Inhibition of Sarcoma 180 by Dilutions of Calvatia gigantea Extracts*

Preparation	Dry wt. of solids, $\mu\text{g./ml.}$	Effect*	Weight change in grams†	Deaths
Aqueous extract of sporophore	475	$\pm^-$	-3.0/-2.0	0
	238	$\pm^-$	-1.5/-2.0	0
	119	$\pm^-$	-2.0/-2.0	0
	60	$\pm^-$	-1.5/-2.0	0
	30	—	-0.5/-2.0	0

\* — = tumor diameter larger than 75 per cent of diameter of control tumor,  $\pm^-$  = tumor diameter 51 to 75 per cent of diameter of control tumor,  $\pm^+$  = tumor diameter 26 to 50 per cent of diameter of control tumor, + = tumor diameter 25 per cent or less of diameter of control tumor.

† Average weight gain or loss for the treated mice in one week/average weight gain or loss for the control mice.

to 49 per cent in comparison with the growth of tumors of untreated animals at levels of approximately 475 to 60  $\mu\text{g./ml.}$  (table I). For investigation of the nature of the active principle ethanol or acetone precipitation was used. Both solvents precipitated some active material at concentrations of 30 and 50 per cent, but the best separation was obtained when either acetone or ethanol were used at 70 to 75 per cent of the total volume. It can be seen in table II that a 30 per cent precipitate inhibited tumor growth by more than 75 per cent when given at a level of 360  $\mu\text{g./ml.}$  Seventy per cent precipitates showed retardation at 29  $\mu\text{g./ml.}$ , while the supernatant liquid exhibited strong to mild inhibition at levels ranging from 450 to 29  $\mu\text{g./ml.}$

Heat stability is limited. When an aqueous preparation is heated to 100 C. a small amount of activity is retained; such preparations were active at the level of about 9 mg./ml. When boiled for 10 minutes the material loses its activity. Treatment with ion exchange resins indicated that some of the activity was either destroyed or adsorbed; aqueous extracts shaken with either Dowex 1-X10 or 50-X4 resin for one hour retained mild activity at pH 6.0 and 2.0, respectively.

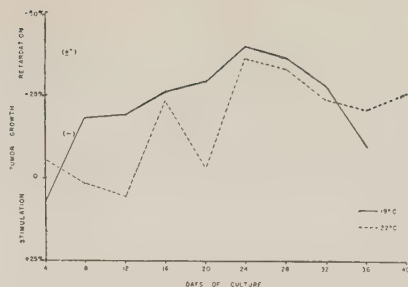
Mycelial transfers from fresh sporophores to solid media were successful. Such transfers were made of almost all the 300 sporophores that were under investiga-

TABLE II  
*Inhibition of Sarcoma 180 by Fractions of Calvatia gigantea Extracts*

Preparation	Dry wt. of solids, $\mu\text{g./ml.}$	Effect*	Weight change in grams*	Deaths
1. Ethanol precipitate (30 per cent) of aqueous extract of sporophore, dissolved in water	360	+	-3.0/-1.5	0
2. Ethanol precipitate (70 per cent) of supernatant liquid of no. 1, dissolved in water	180	$\pm^+$	-2.0/-1.5	0
3. Acetone precipitate (70 per cent of final volume, preceded by 30 per cent) of aqueous extract of sporophore, dissolved in water	225	$\pm^+$	-4.5/-1.0	1
	113	$\pm^-$	-2.5/-1.0	1
	57	$\pm^-$	-1.0/-1.0	0
	29	$\pm^-$	+0.5/-1.0	0
4. Supernatant liquid of no. 2, ethanol evaporated, residue dissolved in water	450	+	-3.5/-2.5	0
	113	$\pm^+$	-3.5/-2.5	0
	57	$\pm^-$	-1.0/-2.5	0
	29	$\pm^-$	-1.0/-2.5	0
	15	—	-1.0/-2.5	0

\* See footnotes in table I.

FIG. 1. The effect on mouse sarcoma 180 of *Calvatia gigantea* preparations made after different growth periods in shaken flasks at 19 and 22 C. is illustrated.



tion. These cultures are being maintained. When growth on agar plates had reached the desired size, flasks with liquid media were inoculated with a suspension of the hyphal material under standardized conditions. These cultures were grown on a shaker usually at 20 to 25 C. After given intervals mycelium and culture broth were removed and tested against sarcoma 180. Figure 1 shows two curves representing the elaboration of the active principle by one of the strains at two different temperatures. Other strains showed different curves under identical conditions. At both temperatures indication of activity occurred on the sixteenth day and the presence of a tumor-inhibitory substance was evident on the twenty-fourth day; however, the curve shows two conspicuous declines in activity only in the 22 C. experiment. These declines may be due to a temperature-dependent cycle in the elaboration of the principle.

Although in the case shown the mycelium had been blended together with the culture broth, separation of them disclosed that activity is present in both mycelium and culture medium. In another experiment the first active preparation was obtained after 20 days of culture; the activity resided in the culture liquid. Subsequent tests, with the exception of the 32 day sample, show this fraction to be the carrier of a tumor inhibitor. In addition, starting with the 24 day samples, tumor-inhibitory principles are found in the aqueous extract of the mycelium; the 70 per cent acetone or ethanol precipitate of this extract; the 70 per cent acetone or ethanol precipitate of the culture liquid; and the supernatant of the blend of mycelium with culture liquid. On the fortieth day, activity also appeared in the 70 per cent precipitate of the blend of mycelium and culture medium.

## DISCUSSION

The results presented prove that tumor inhibitors can be obtained from fresh and frozen sporophores as well as from shake cultures of sporophore-derived mycelia. So far, there is no indication that the potency of a sporophore preparation can be correlated with the elaboration of the active principle *in vitro*. Activity in both cases appears to be governed by a set of environmental conditions the complete elucidation of which will take time. There is experimental evidence that the isolates from the different sporophores resulted in cultures with greatly different physiological characteristics, among which, to us, the mode of elaborating the active principle is the most interesting one. Several of the isolates were subjected to beta-ray irradiation by means of an electron beam generator; this has resulted in the development of modified strains some of which are superior to their sources in the production of the active principle.

This report is a preliminary one. Work on several of the problems involved is in progress. The results will be published in detail elsewhere.

## SUMMARY

Tumor inhibiting substances have been obtained from fresh and frozen sporophores of several species of the genus *Calvatia*, primarily from *C. gigantea* (Pers.) Lloyd. The successful culture of this organism in vitro and the production of the tumor inhibiting principles in fermentation cultures is reported.

## BIBLIOGRAPHY

1. LUCAS, E. H.; RINGLER, R. L.; BYERRUM, R. U.; STEVENS, J. A.; CLARKE, D. A., AND STOCK, C. C.: Tumor inhibitors in *Boletus edulis* and other holobasidiomycetes, *Antib. & Chemo.* 7:1-4, 1957.
2. STOCK, C. C.: Experimental cancer chemotherapy. *In: Advances in Cancer Research* 2:425-492, 1954.

# Actinobolin, a New Broad-Spectrum Antibiotic

## Origin and Biological Evaluation

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We wish to announce a new antibiotic to be called "actinobolin" and to report on its biologic activity. Actinobolin was obtained<sup>1,2</sup> from submerged, aerated broth cultures of a *Streptomyces*\* designated P-D 05000. Haskell and Bartz<sup>1</sup> have isolated actinobolin from culture filtrates by adsorption-elution methods. The substance is a hydrophilic, amphoteric, water-soluble base of relatively low molecular weight. It readily forms water-soluble crystalline salts with some acids and it chelates with iron, aluminum, and other metal ions. Actinobolin inhibits the growth of various gram-positive and gram-negative bacteria. It has also been found at Sloan-Kettering Institute to be effective at relatively high concentrations in repressing the growth of a number of transplantable mammalian neoplasms in vivo.<sup>3-6</sup> It is relatively low in toxicity. Oral doses are poorly absorbed.

### FERMENTATION AND ASSAY

The *Streptomyces* grows well in various media, in some of which little antibacterial activity appears. This dependence of antibacterial activity on medium composition is illustrated in table I.

Actinobolin is assayed by a paper disc-tray, agar-diffusion method similar to those used for viomycin,<sup>7</sup> with the following modifications. The test organism used is *Sarcina lutea* PCI 1001W. A cell suspension is adjusted so that a  $\frac{1}{10}$  dilution gives 16 per cent light transmission in a Coleman Jr. spectrophotometer, Model 6A, at a wave length of 555 m $\mu$ . A  $\frac{1}{600}$  dilution of the adjusted suspension is made in melted and cooled Penassay seed agar (Difco) and a 25 ml. aliquot is poured into each tray. No basal layer is employed in this test. Incubation is at 37 C. for 18 hours. The original working standard was a beer with an activity of 40 *S. lutea* dilution units/ml. Later, a crude concentrate assaying 29 units/mg. was established as the working standard. This concentrate was eventually replaced by crystalline actinobolin sulfate assaying 80 to 90 units/mg. The standard and samples, including body fluids, are diluted in pH 7.8 phosphate buffers in such a manner that they are in 0.1 M buffer solution.

### ANTIBACTERIAL ACTIVITY

Shaken flask culture centrifugates or filtrates caused zones of inhibition on Trypticase soy agar (BBL) medium seeded with *Bacillus subtilis*, *Escherichia coli*,

\* The *Streptomyces* culture stemmed from a Georgia soil isolate obtained in Paul R. Burkholder's laboratory at the University of Georgia. The isolate was one of many that Dr. Burkholder sent to one of us (J. E.) for antibiotic screening.

TABLE I  
Antibacterial Titer of Media A and B

Ingredients	Concentration, per cent (w/v)		Incubation time, hours	Antibacterial titer, units/ml.	
	A	B		A	B
Glucose monohydrate, commercial	0.5	—	72	2.3	39.0
Glycerol U.S.P.	—	1.0	96	2.1	43.0
Casein, acid hydrolyzed	0.3	—	110	1.2	21.3
Acetone-butanol fermentation residue	0.25	0.5	Conditions: spore inoculum 250 ml. medium/L. flask 28 C. 160 r.p.m. rotary shaker		
Yeast, brewers; debittered	0.1	1.0			
Sodium chloride	0.5	0.5			
Sodium hydroxide to pH 7.5	—	—			
Calcium carbonate	0.1	0.1			

TABLE II  
The in Vitro Antibacterial Spectrum of Actinobolin

Bacteria	Additional designation	50% inhibition by crystalline sulfate, $\mu\text{g.}/\text{ml.}$	Minimal inhibitory concentration of aluminum complex, $\mu\text{g.}/\text{ml.}$	
			Complete inhibition	Partial inhibition
Turbidimetric Inhibition Tests				
<i>Bacillus firmus</i>	P-D M1539	16.0		
<i>Staphylococcus aureus</i>	ATCC 6538	2.0		
<i>Staphylococcus aureus</i>	P-D 04984	2.0		
<i>Staphylococcus aureus</i>	P-D 04988	2.5		
<i>Streptococcus</i> sp.	ATCC 9854	8.9		
<i>Aerobacter aerogenes</i>	P-D 0126	8.3		
<i>Agrobacterium tumefaciens</i>	P-D M1221	16.0		
<i>Escherichia coli</i>	P-D 04863	3.5		
<i>Klebsiella pneumoniae</i>	ATCC 10031	8.3		
<i>Proteus vulgaris</i>	P-D 04736	>50.0		
<i>Proteus vulgaris</i>	S & D 1810	2.9		
<i>Salmonella schottmuelleri</i>	P-D 01180	3.8		
<i>Salmonella typhosa</i>	P-D 02481	4.5		
<i>Shigella paradyserteriae</i>	P-D 02904	0.91		
<i>Shigella sonnei</i>	ATCC 11060	0.81		
<i>Vibrio comma</i>	NIH 35A3	0.53		
Broth Dilution Tests				
<i>Corynebacterium diphtheriae</i>	P-D 036		6.25	3.1
<i>Diplococcus pneumoniae</i>	SVI		6.25	1.6
<i>Staphylococcus aureus</i>	Smith		3.1	1.6
<i>Staphylococcus aureus</i>	ATCC 6538		12.5	6.25
<i>Staphylococcus aureus</i>	TTC-R from ATCC 6538		6.25	3.1
<i>Staphylococcus aureus</i>	PEN-R & ERM-R from P-D 04947		3.1	0.8
<i>Streptococcus faecalis</i>	P-D "V"		>100.0	—
<i>Streptococcus pyogenes</i>	C203		3.1	0.4
<i>Streptococcus salivarius</i>	P-D 04150		6.25	1.6
<i>Mycobacterium phlei</i>	ATCC 356		50.0	—
<i>Mycobacterium tuberculosis</i> var. <i>hominis</i>	H <sub>37</sub> Rv		100.0	—
<i>Aerobacter aerogenes</i>	P-D 0126		25.0	12.5
<i>Brucella suis</i>	Huddleson 1772		1.6	—
<i>Escherichia coli</i>	P-D 04863		25.0	12.5
<i>Klebsiella pneumoniae</i>	AD		50.0	—
<i>Neisseria catarrhalis</i>	P-D 03447		1.6	0.8
<i>Pasteurella multocida</i>	P-D 02855		0.8	—
<i>Proteus vulgaris</i>	S & D 1810		100.0	50.0
<i>Pseudomonas aeruginosa</i>	P-D 01925		>100.0	—
<i>Salmonella typhimurium</i>	V31		100.0	50.0
<i>Salmonella typhosa</i>	P-D 02481		12.5	6.25
<i>Shigella dysenteriae</i>	P-D 01339		25.0	12.5

TABLE III  
*Antibacterial Activity of Actinobolin in Mice*

Infecting organism	Agent	Dosage,* mg./Kg./day	Route	Survivors,† per cent
<i>Staph. aureus</i> Smith	—	—	—	0
(1 day treatment)	Sulfadiazine	50	Oral	100
(1 day treatment)	Actinobolin	250	Oral	10
(1 day treatment)	Actinobolin	500	Oral	20
(1 day treatment)	Actinobolin	1000	Oral	90
(1 day treatment)	Actinobolin	125	Subcutaneous	40
(1 day treatment)	Actinobolin	250	Subcutaneous	100
(1 day treatment)	Actinobolin	500	Subcutaneous	90
(1 day treatment)	Actinobolin	1000	Subcutaneous	90
<i>Str. pyogenes</i> C203	—	—	—	0
(1 day treatment)	Sulfadiazine	50	Oral	40
(1 day treatment)	Actinobolin	250	Oral	40
(1 day treatment)	Actinobolin	500	Oral	20
(1 day treatment)	Actinobolin	1000	Oral	60
(1 day treatment)	Actinobolin	125	Subcutaneous	70
(1 day treatment)	Actinobolin	250	Subcutaneous	90
(1 day treatment)	Actinobolin	500	Subcutaneous	70
(1 day treatment)	Actinobolin	1000	Subcutaneous	80
<i>Klebsiella pneumoniae</i> AD	—	—	—	10
(2 days treatment)	Sulfadiazine	100	Oral	30
(2 days treatment)	Actinobolin	250	Oral	30
(2 days treatment)	Actinobolin	500	Oral	30
(2 days treatment)	Actinobolin	1000	Oral	0
(2 days treatment)	Actinobolin	125	Subcutaneous	30
(2 days treatment)	Actinobolin	250	Subcutaneous	40
(2 days treatment)	Actinobolin	500	Subcutaneous	80
(2 days treatment)	Actinobolin	1000	Subcutaneous	80

\* Two divided doses a day, six hours apart.

† Survival values are based on observations made 7 to 14 days postinfection; groups of 10 mice were used at each dose level.

*Mycobacterium phlei*, *Proteus vulgaris*, *Salmonella typhimurium*, *S. lutea*, and *Staphylococcus aureus*.

The in vitro activity of actinobolin is summarized in table II.

In studies with two strains of staphylococci and one strain of hemolytic *Streptococcus*, it was observed in broth transfer tests that resistance to actinobolin developed readily. Cross resistance with the principal antibiotics in clinical use today was not found, and staphylococci resistant to these other antibiotics were uniformly sensitive to actinobolin.

Actinobolin was tested for antibacterial activity in mice against a variety of acute experimental infections. Previously described test procedures were used.<sup>8</sup> Typical experimental results obtained with three sensitive strains are summarized in table III. Actinobolin was essentially ineffective at doses as high as 1000 mg./Kg./day either orally or subcutaneously against experimental mouse infections with *Diplococcus pneumoniae* SVI or *P. vulgaris* 1810. Against a *Sal. typhimurium* V31 infection, no prolongation of survival time occurred with oral doses up through 1000 mg./Kg./day; however, subcutaneous doses of 125 through 1000 mg./Kg./day exhibited a small degree of activity. Against *Mycobacterium tuberculosis* var. *hominis* H<sub>37</sub>Rv in mice, actinobolin given subcutaneously up through dosages of 1000 mg./Kg./day for five days was not effective, nor did such doses improve the activity of streptomycin.

Actinobolin when given intraperitoneally twice daily for four days inhibited *Plasmodium lophurae* in chicks. At doses of 125 mg./Kg./day a 50 per cent reduction in cells parasitized was observed. However, increasing the dose to 250 and 500 mg./Kg. resulted only in 64 and 65.5 per cent reductions, respectively, of cells parasitized. In this test, quinine is approximately 10 times more active than actinobolin. The techniques employed have been previously described.<sup>9</sup>

Actinobolin was tested for therapeutic activity in albino mice (Webster strain, females) infected with *Schistosoma mansoni* (Puerto Rican strain). The mice were infected intraperitoneally with 75 cercariae and held six weeks to allow the worms to mature; treatment consisted of two daily intraperitoneal doses for 10 consecutive days; the mice were held an additional 18 days and sacrificed. Therapeutic effects were determined by counting worms in portal-mesenteric veins and in the liver, using the criteria of distribution of worms between liver and veins; presence of dead worms in the liver; and total number of worms relative to parallel untreated controls. At dosages as high as 500 mg./Kg./day actinobolin exhibited no anti-schistosomal activity.

Actinobolin was ineffective against intestinal helminthiases in mice. Mice experimentally infected with 20 larvae of *Nematospiroides dubius* and carrying natural infestations of *Syphacia obvelata*, *Aspicularis tetraptera*, and *Hymenolepis nana* were not cleared of worms by oral doses as high as 2000 mg./Kg./day.

Actinobolin was found to be inactive in vitro at 500  $\mu$ g./ml. versus *Entamoeba histolytica* and at 200  $\mu$ g./ml. versus *Trichomonas vaginalis*.

#### TOXICOLOGICAL STUDIES

Toxicity studies in mice, rats, dogs, and monkeys indicated that actinobolin is relatively nontoxic at high dosages for these animals (see table IV).

TABLE IV

*Toxicity Studies on Actinobolin*

Type of experiment	Route	Animal and no. used	Dose range, mg./Kg./day	No. of animals /dose	Length of dosing	Length of observation	Results
Acute (1 dose)	Intravenous	Mouse 75	500 to 1000	15 to 20	1 day	1 week	LD <sub>50</sub> = 800 $\pm$ 27* mg./Kg.
Acute (1 dose)	Intravenous	Rat 85	500 to 1750	5 to 20	1 day	1 week	LD <sub>50</sub> = 1550 $\pm$ 26* mg./Kg.
Chronic 5 days/wk.	Subcutaneous	Rat 40	125 to 500	10	6 weeks	6 weeks	62.5 mg./Kg. twice daily best tolerated
Chronic 5 days/wk.	Intramuscular	Dog 6	25 to 100	2	6 weeks	6 weeks	Dose related vomiting and salivation after injection (see text)
Chronic 5 days/wk.	Intramuscular	Monkey 6	25 to 200	2	6 weeks	6 weeks	No consistent drug related toxicity (see text)

\* Standard error, by method of Miller and Tainter.

In acute intravenous toxicity trials in mice it was observed that animals given 500 mg./Kg. remained normal. At doses of 750 mg./Kg. and higher, the mice showed severe incoordination with occasional tonic convulsions and dyspnea immediately after injection; surviving animals appeared normal five to fifteen minutes after injection. Deaths occurred within two minutes after injection, associated with respiratory failure.

Rats receiving single intravenous doses of 500 to 875 mg./Kg. remained normal after injection. Doses of 1000 and 1250 mg./Kg. caused transient, moderate to severe incoordination with slight tonic convulsions and occasional trembling; surviving animals showed depression in five minutes and complete recovery within 30 minutes. Animals given 1500 and 1750 mg./Kg. showed severe incoordination, flaccid prostration, and slight tonic convulsions immediately after injection. These reactions subsided to depression in five minutes and complete recovery followed within 30 minutes in surviving animals. Deaths were associated with respiratory failure and occurred 1 to 10 minutes after drug administration.

The effect of repeated subcutaneous administration of actinobolin to albino rats for six weeks was investigated. It was found that 62.5 mg./Kg. twice a day, five days a week for six weeks, was well tolerated with no food intake depression and only a slight depression in weight gain. Rats receiving 125 or 250 mg./Kg. twice a day for the test period showed slight to moderate depression in food intake and marked to severe depression in weight gain. All of the drug-treated animals showed soft stools during the second week of treatment and in those animals receiving 250 mg./Kg. twice a day the condition was accompanied by abdominal distention. The condition persisted and reached a maximum during the third and fourth weeks, after which it subsided at all dose levels during the fifth and sixth weeks. Undoubtedly this intestinal disorder was partially responsible for the weight gain depression which occurred. Total and differential white cell counts, in addition to hematocrit and hemoglobin determinations, were performed initially and at the end of the experiment on all animals. All values remained within the normal range.

Six beagle dogs received actinobolin intramuscularly in doses of 25, 50, and 100 mg./Kg./day for six weeks. The only toxic reaction was salivation and vomiting, the severity and duration of which was directly related to the dosage, and which subsided shortly after injection. No significant change occurred in body weights and hematological values remained within the range of normal variation.

Pairs of Rhesus monkeys were given actinobolin intramuscularly once daily, five days a week, in doses of 50, 100, and 200 mg./Kg./day for a period of six weeks. The test was initiated at doses of 25, 50, and 100 mg./Kg./day but, as no toxic reactions were noted during the first week, the low dose animals (25 mg./Kg./day) were raised to 200 mg./Kg./day in an attempt to reach a toxic level. Fifty mg./Kg. for six weeks was well tolerated; no significant changes in body weight, hematological values or other toxic indications were noted. One monkey receiving 100 mg./Kg./day also remained symptom free and in good condition. The second monkey receiving 100 mg./Kg./day developed a diarrhea that resulted in a terminal weight loss of 0.60 Kg. One monkey receiving 200 mg./Kg./day for the six week period developed a generalized scaling of the skin but showed no significant changes in body weight or hematological values. A second monkey tolerated this dose for two weeks at which time he was sacrificed because of a positive tuberculin reaction. In view of the fact that one monkey tolerated a dose of 200 mg./Kg. daily for a period of six weeks without evident gastrointestinal abnormalities, it is doubtful that the diarrhea encountered in one of the monkeys at 100 mg./Kg. daily is drug

TABLE V  
*Actinobolin in Rat Blood Plasma*

Time after dose, minutes	Dose, mg./Kg.	Actinobolin in plasma, $\mu$ g./ml.	
		Following subcutaneous administration	Following oral administration
30	25	0	0
	50	31	0
	100	64	0
60	25	0	0
	50	0	0
	100	0	0
120	25	0	0
	50	0	0
	100	0	0

related. However, the gastrointestinal effects of actinobolin which were encountered in the rat and dog suggest that the middle-dose monkey which exhibited diarrhea may have been more sensitive in responding to the drug than the other monkeys in the study.

Liver, kidney, and bone marrow biopsy specimens from all dogs and monkeys at all dose levels were examined prior to initiation of the chronic studies. At the end of the study, all major tissues and organs from the high-dose and control groups of rats, and from high- and middle-dose monkeys and dogs were studied microscopically following gross examination at complete autopsy. Liver, kidney, and bone marrow biopsy specimens from the low-dose dogs and monkeys were examined at the end of the study in lieu of a complete autopsy procedure.

The major findings in all species consisted of morphological changes in the glandular derivatives of the gastrointestinal tract, none of which constitute irreversible or significant tissue damage. These changes included mild hydropic vacuolization in the liver, pancreas, and salivary glands. An increase in granularity was noted in the Panneth cells of the rat and monkey. In addition, mild hydropic vacuolization was noted in the renal tubules of the rat and monkey at the highest dose, without significant inflammatory or degenerative changes. The bone marrow of all animals remained normal in all respects.

TABLE VI  
*Urinary Excretion of Actinobolin by Rats*

Collection period, hours	Dose, mg./Kg.	Per cent of dose in urine	
		Following subcutaneous administration	Following oral administration
0-7	25	44	<4
	50	38	2
	100	49	2
7-24	25	0	0
	50	13	0
	100	0	0
0-12	25	36	0
	50	42	3
	100	44	4
0-18	25	28	0
	50	32	<5
	100	39	0

The studies indicate that actinobolin may be administered intramuscularly in amounts up to 2 ml. of a 25 per cent solution without significant local irritation. A 50 per cent concentration, however, results in a predictable slight to moderate injury.

The concentration of actinobolin in the blood and urine of rats following oral and subcutaneous administration was determined by microbiological assay using *S. lutea* PCI 1001W. Albino rats weighing 130 to 140 Gm. were given 25, 50, or 100 mg./Kg. actinobolin by oral intubation or by subcutaneous injection. For the blood studies, individual animals were sacrificed 30 minutes, 1 hour, and 2 hours after dosage, and heart blood was taken for analysis. Urine samples were collected for 7 and 24 hour periods on one group of the rats, for a 12 hour period on a second group and for an 18 hour period on a third. The results of the blood and urine studies are summarized in tables V and VI.

Following oral administration little or no actinobolin was found in the blood and urine. Following subcutaneous administration, however, high levels of actinobolin were found in the urine in spite of the fact that no persistent levels were found in the blood, indicating that the material was rapidly cleared from the blood and accumulated in the urinary tract.

#### SUMMARY

Actinobolin was obtained from aerated broth cultures of a *Streptomyces*. An agar diffusion assay using *S. lutea* as the test organism was employed. Actinobolin was found to be a potent inhibitor of various gram-positive and gram-negative bacteria in vitro and relatively high doses protected mice against experimental staphylococcal, streptococcal, and *Klebsiella* infections. Essentially complete protection against an acute infection with *Staph. aureus* Smith was conferred by a single day's treatment with 1000 mg./Kg./day by mouth or 500 mg./Kg./day subcutaneously. However, actinobolin was essentially ineffective against experimental mouse infections with *D. pneumoniae* SVI, *P. vulgaris* 1810, *Sal. typhimurium* V31, or *Myco. tuberculosis* H<sub>37</sub>Rv. Gram-positive cocci developed resistance to actinobolin in vitro but cross resistance with the principal clinically employed antibiotics did not occur. Actinobolin inhibited *P. lophurae* infections in chicks but was only approximately one-tenth as active as quinine. *S. mansoni* infections in mice were not affected by actinobolin administered intraperitoneally at dosages as high as 500 mg./Kg./day. Actinobolin was ineffective against intestinal helminthiases in mice and against *E. histolytica* and *T. vaginalis* in vitro.

High doses were well tolerated by mice, rats, dogs, and monkeys. Acute intravenous LD<sub>50</sub> values were approximately 800 and 1550 mg./Kg. in mice and rats, respectively. No irritation occurred at injection sites with a 25 per cent solution and no evidence of significant irreversible organ toxicity was observed. Actinobolin appeared to be poorly absorbed following oral administration. Parenteral doses were followed by substantial but transitory blood levels and sustained high urine levels.

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#### BIBLIOGRAPHY

1. HASKELL, T. H., AND BARTZ, Q. R.: Actinobolin, a new broad-spectrum antibiotic. Isolation and characterization. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 505.
2. FUSARI, S. A., AND MACHAMER, H. E.: Actinobolin, a new broad-spectrum antibiotic. Pilot plant studies. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 510.
3. BURCHENAL, J. H.; HOLMBERG, E. A. D.; REILLY, H. C.; HEMPHILL, S., AND REPERT, J. A.: The effects of actinobolin on transplanted mouse leukemias. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 528.
4. SUGIURA, K., AND REILLY, H. C.: The effects of actinobolin on a spectrum of tumors. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 522.
5. TELLER, M. N.; MERKER, P. C.; PALM, J. E., AND WOOLLEY, G. W.: The effects of actinobolin on the growth of human tumors H.S.#1 and H.Ep.#3 in the rat. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 518.
6. MERKER, P. C., AND WOOLLEY, G. W.: The effects of actinobolin on human epidermoid carcinoma (H. Ep.#3) growing in the conditioned Swiss mouse. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 515.
7. EHRLICH, J.; IVERSON, W. P., AND KOHBERGER, D.: Agar diffusion methods for the assay of viomycin, *Antib. & Chemo.* 1:211-216, 1951.
8. FISHER, M. W., AND MANNING, M. C.: Synergism between human gamma globulin and chloramphenicol in the treatment of experimental bacterial infections, *Antib. & Chemo.* 7:315-321, 1957.
9. MCCARTHY, D. A.; BAYLES, A., AND THOMPSON, P. E.: The effect of azaserine against *Plasmodium lophurae* in chicks and attempts to antagonize this effect with metabolites, *J. Parasitol.* 43:283-290, 1957.

# Actinobolin, a New Broad-Spectrum Antibiotic

## Isolation and Characterization

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A new broad-spectrum antibiotic, for which the generic name actinobolin has been proposed, has been biologically characterized by Pittillo et al.<sup>1</sup> This paper deals with the isolation and chemical characterization of this substance.

Actinobolin has been isolated as crystalline acetate and sulfate salts and as the amorphous free base. Microanalyses of these salts as well as the biologically inactive N-acetyl derivative support the empirical formula  $C_{13}H_{20-22}N_2O_6$  for the free base. Actinobolin base as well as its salts are very soluble in water and cannot be satisfactorily extracted from aqueous solution with any of the common (immiscible) organic solvents. Hydrogen binding titration curves showed the compound to be amphoteric with a basic function of  $pK_a$  7.5 and a weakly acidic (enolic) function of  $pK_a$  8.8. Molecular weight values from these titrations were 368 for the acetate and 352 for the half-sulfate. One nitrogen atom per molecule of actinobolin was liberated by the Van Slyke procedure while the remaining nitrogen atom was non-basic as evidenced by a perchloric acid in glacial acetic acid titration.

Actinobolin sulfate shows a single characteristic ultraviolet absorption maximum at  $\lambda$  263  $m\mu$  ( $a = 26.6$ ) in 0.1 *N* hydrochloric acid, at  $\lambda$  264  $m\mu$  ( $a = 25.3$ ) in 0.1 *M* phosphate buffer at *pH* 7.0, and at  $\lambda$  288  $m\mu$  ( $a = 40.6$ ) in 0.1 *N* sodium hydroxide solution (fig. 1). In aqueous solution the antibiotic shows maximum stability at *pH* 3.0, such solutions retaining their microbiological potencies at 37 C. for seven days. Actinobolin is quite unstable in aqueous solutions at *pH* values of 7 or higher. Approximately 70 per cent of its activity was destroyed at *pH* 7.0 after 72 hours at room temperature. Decreases in biological potencies can be followed quite readily by ultraviolet absorption measurements, since the absorbency of the maximum at  $\lambda$  263 to 264  $m\mu$  exhibited a corresponding decrease with biological potency with no subsequent wavelength shift. The infrared absorption spectrum of the crystalline sulfate is shown in figure 2. The sulfate shows strong OH, NH (2.90  $\mu$ ), unidentified carbonyl (5.90  $\mu$ ), and amide (6.00, 6.18  $\mu$ ) functions. It also exhibits broad absorption in the 3.0 to 3.8  $\mu$  region which is characteristic for protonated amines.

Actinobolin gives a purple color with ninhydrin, a red-orange color with the Pauli diazo reagent, a red color with ferric chloride, and decolorizes potassium permanganate in the cold. It reduces Fehling's solution and gives positive Folin-Ciocalteu and iodoform tests. It is negative to the Molisch, Ehrlich (dimethylaminobenzaldehyde), and Elson-Morgan tests. A reactive carbonyl grouping was not detected by hydroxylamine titration. It absorbed no hydrogen with Adams' or Raney nickel catalysts in either acetic acid or ethanol. Oxidation of actinobolin with iodine in sodium bicarbonate solution resulted in the rapid removal of 8 electrons (10 minutes) with concurrent destruction of its ultraviolet absorption and biological activity.

The antibiotic is a strong complexing agent for the group III elements, in particular iron and aluminum. Isolated concentrates of actinobolin from carbon and Decalso columns showed deep red colorations due to complexed iron extracted from

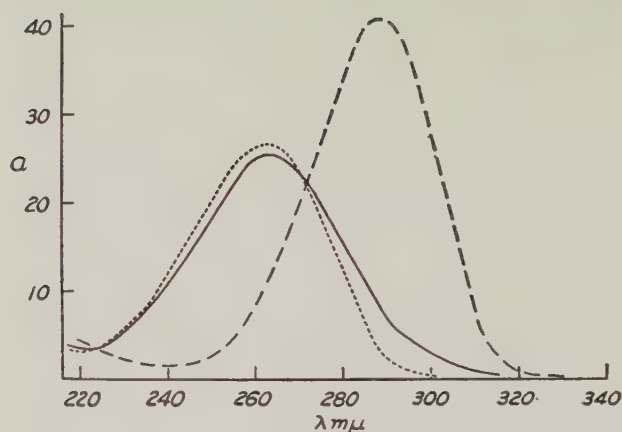


FIG. 1. Ultraviolet absorption spectrum of actinobolin sulfate is shown --- 0.1 *N* hydrochloric acid; — 0.1 *M* pH 7.0 aqueous phosphate buffer; ··· 0.1 *N* sodium hydroxide.

the adsorbents. Removal of iron from the concentrates was accomplished by cupferron in *n*-butanol-chloroform extraction. In a few instances some difficulty was experienced in crystallizing preparations of the sulfate salt due to the bound aluminum picked up from the aluminum silicate (Decalso) adsorbent. This was overcome by the removal of aluminum either by oxine precipitation or preferential adsorption on Dowex 50 columns. The actinobolin-aluminum complex was prepared by mixing ethanolic solutions of the antibiotic and aluminum chloride. The amorphous precipitate, which separated, was microbiologically active (70 *Sarcina lutea* units/mg.), showed no wave length shift in its ultraviolet absorption maximum from the parent compound, but possessed a considerably higher optical rotational value in water ( $[\alpha]_D^{26} = +138^\circ$ ) than the sulfate salt ( $[\alpha]_D^{26} = +54.5^\circ$ ).

Paper chromatographic examination of actinobolin concentrates and crystalline salt preparations using a wide variety of solvent systems showed only one microbiologically active zone.

The preparation of actinobolin from filtered beer consisted essentially of three steps. The first involved adsorbing the material onto Darco G-60 and eluting with aqueous acetone. The process may be performed by either the batchwise or column methods and a recovery of 60 to 70 per cent was usually obtained.

The next step consisted of passing the carbon eluates over a cation exchange adsorbent. The exchanger used in this study was the synthetic sodium aluminum silicate known as Decalso. As the actinobolin was adsorbed on the column it formed a light pink band due to iron present in the Decalso and the antibiotic solu-

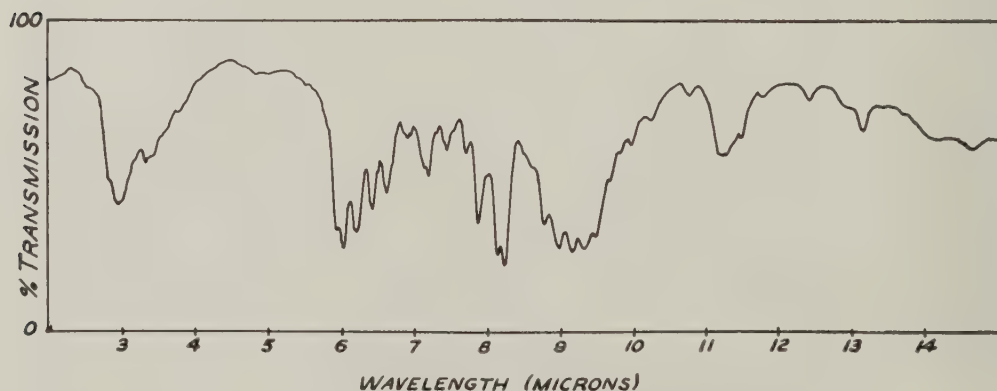


FIG. 2. Infrared absorption spectrum of crystalline actinobolin sulfate in compressed potassium bromide is given.

tion was introduced until the column was almost completely colored. The activity was recovered by elution with acidic aqueous alcohol mixtures.

The final step consisted of passing the concentrated Decalso eluates over a Darco G-60-Celite 545 column followed by aqueous acetone elution. The actinobolin breakthrough was quite readily detected by the appearance of a deep red color which gradually diminished as the elution progressed. After pH adjustment with dilute sulfuric acid and removal of the acetone in vacuo, the iron was removed by extraction with a 0.02 *M* cupferron in 50:50 *n*-butanol-chloroform solution. Lyophilization of the aqueous layer yielded actinobolin sulfate of 80 to 90 per cent purity which could then be crystallized from aqueous ethanol.

The antibiotic free base was readily prepared by passing solutions of the crystalline sulfate over a column containing the anion exchange resin Amberlite IR-45 in the hydroxyl form and freeze drying the effluent and washings. The acetate was prepared by dissolving the base in absolute ethanol, adding an excess of acetic acid, and precipitating with ethyl acetate. Recrystallization from absolute ethanol gave the acetate as white needles which melted at 263 to 266 C. after softening at about 130 C. and resolidifying at 145 C. The salt had an  $[\alpha]_D^{26}$  of  $+58^\circ$  in water. A perchloric acid in glacial acetic acid titration gave a value of 3.91 per cent nitrogen and a neutral equivalent of 357. A Kjeldahl nitrogen value of 7.52 per cent was obtained.

Treatment of the crystalline acetate or free base with acetic anhydride gave a biologically inactive N-acetyl derivative. The product melted with decomposition at 254 to 255 C. and showed only one ionizable group ( $pK_a$  8.4) by titration.

Toxicity studies in several species of experimental animals indicated that actinobolin is relatively nontoxic.<sup>1</sup> The antibiotic, in high dosage, protected mice against streptococcal, staphylococcal, and *Klebsiella* infections by both oral and subcutaneous routes of administration. The latter method of treatment was more effective than the former.<sup>1</sup> Actinobolin also has been found to be active against certain transplantable mammalian neoplasms in vivo.<sup>2-5</sup>

#### EXPERIMENTAL

*Assay.* Antibiotic potency was determined by using a paper disc-tray, agar-diffusion method and *S. lutea* PCI 1001W as the assay organism.<sup>1</sup> A crude actinobolin concentrate was used as the standard and crystalline preparations of the acetate and sulfate assayed 80 to 90 units/mg. of this standard.

*Carbon Adsorption.* Stirred jar harvested fermentation beer (91 liters) was adjusted to pH 2.0 with sulfuric acid, slurried with 2 per cent w/v Celite 545, and filtered. The filtered beer, which assayed 12.6 million *S. lutea* units, was adjusted to pH 4.0 with alkali and stirred with 6 Kg. of Darco G-60 for 30 minutes. Celite 545 (2.25 Kg.) was then added and the mixture filtered through a 12 inch plate and frame Schriver filter press which had been precoated with 640 Gm. of Celite. The carbon cakes were then washed with 20 liters of water. The spent beer and water washings assayed less than 2 *S. lutea* units/ml. The press was then eluted with four 15 liter portions of 40 per cent aqueous acetone which removed a total of 5.85 million units. Elution with an additional 40 liters of from 30 to 40 per cent aqueous acetone removed another 2.64 million units for a total recovery of 67 per cent. The combined eluates were adjusted to pH 3.5 with sulfuric acid and concentrated in vacuo to a volume of 22 liters.

*Decalso Chromatography.* Decalso (8 liters) was slurried with water and treated with hydrochloric acid until the pH remained at 6.0 for 30 minutes. It was then packed into a glass pipe (6 inch internal diameter) and rinsed with water. The com-

bined carbon eluates from the previous step were then passed over the adsorbent and the column rinsed with 30 liters of water. The column assumed a pink coloration down to about 3 inches from the bottom. Leakage amounted to less than 3 per cent. Actinobolin was then eluted with 5 per cent aqueous acetic acid containing approximately 10 per cent ethanol (20 liters). The first 16 liters of eluate following the acid breakthrough contained 6.48 million *S. lutea* units. An additional 5.5 liters collected increased the recovery to 6.9 million units (81 per cent). The combined eluates were then concentrated in vacuo to a volume of 8.5 liters.

*Darco G-60-Celite Chromatography.* Darco G-60 (2.5 Kg.) and Celite 545 (2.5 Kg.) were slurried together in water, pumped into a glass column (6 inch internal diameter), and rinsed with water under 6 lb. pressure. The actinobolin solution obtained from the Decalso column (6.9 million units) was passed through the column and rinsed with 35 liters of water. The amount of actinobolin leakage was less than 10 per cent. Actinobolin was then eluted with 20 per cent aqueous acetone. A pressure of 6 lb./sq. in. was maintained throughout the chromatographic procedure. The first liter of eluate which appeared at the acetone front was deep red and contained 1.9 million units. The next 1.25 liters contained 1.87 million units and the 8.2 liters which followed contained 1.67 million units. A total recovery of 5.6 million units was obtained in the acetone eluates (81 per cent).

The second eluted fraction (1.25 liters) was adjusted to pH 3.5 with sulfuric acid and concentrated in vacuo to a final volume of 900 ml. A portion (860 ml.) of this solution which had a pH of 3.2 was mixed with 150 ml. of a 0.02 *M* cupferron in 50:50 *n*-butanol-chloroform solution with rapid stirring. The layers were separated and the extraction repeated with an additional 100 ml. of cupferron solution. The second cupferron extract was colorless indicating complete iron removal. The clear light yellow aqueous layer was then extracted two times with 150 ml. portions of chloroform and the aqueous layer readjusted to pH 3.2 with sulfuric acid. The aqueous layer was concentrated in vacuo and dried from the frozen state. Actinobolin sulfate (19.8 Gm.) was obtained as a buff colored solid of approximately 90 per cent purity. Crystallization from 25 ml. of water and 45 ml. of ethanol afforded 13.1 Gm. of colorless crystalline actinobolin sulfate which assayed 80 to 90 *S. lutea* units/mg.  $a = 25.3$  at  $\lambda$  max 264  $m\mu$  in pH 7.0 phosphate buffer.  $[\alpha]_D^{26} + 54.5^\circ$  (*c*, 1 per cent in water).

ANALYTICAL. Calculated for  $(C_{13}H_{20}N_2O_6)_2H_2SO_4 \cdot 2H_2O$ : C, 42.50; H, 6.31; N, 7.63;  $SO_4 =$ , 13.08; mol. wt., 734.75. Calculated for  $(C_{13}H_{22}N_2O_6)_2H_2SO_4 \cdot 2H_2O$ : C, 42.27; H, 6.82; N, 7.58;  $SO_4 =$ , 13.00; mol. wt., 738.78. Found: C, 42.28; H, 6.45; N, 7.49;  $SO_4 =$ , 15.3.\* No ash.

*Actinobolin Free Base.* Actinobolin sulfate (1.0 Gm.) was dissolved in water and passed over a column containing 12 ml. of Amberlite IR-45 (OH-form). The column was rinsed well with water and the combined effluent and washings freeze dried. Actinobolin free base (0.67 Gm.) was obtained as an amorphous white fluffy powder assaying 97 *S. lutea* units/mg.  $a = 28.2$  at  $\lambda$  max 263  $m\mu$  in phosphate buffer pH 7.0.

ANALYTICAL. Calculated for  $C_{13}H_{20}N_2O_6 \cdot \frac{1}{2} H_2O$ : C, 50.48; H, 6.84; N, 9.06. Calculated for  $C_{13}H_{22}N_2O_6 \cdot \frac{1}{2} H_2O$ : C, 50.15; H, 7.45; N, 9.00. Found: C, 50.31; H, 6.88; N, 9.17. Volatile loss at 50 C., 4.09. The dried sample was very hygroscopic.

\* The sulfate was determined by volumetric titration using the tetrahydroxyquinone indicator. The high value is due to chelation of barium ion by actinobolin during the titration.

*Actinobolin Acetate.* A sample of the free base (0.20 Gm.) was dissolved in 0.7 ml. of absolute ethanol and 0.15 ml. of glacial acetic acid added followed by 3 ml. of ethyl acetate. After cooling, the product was collected by filtration, washed with ethyl acetate, and dried in vacuo. The actinobolin acetate obtained (0.21 Gm.) readily afforded white needles upon recrystallization from 2 ml. of absolute ethanol. The product partially melted at 130 C., resolidified at 145 C., and melted with decomposition at 263 to 266 C. Bioassay, 86 *S. lutea* units/mg.  $a = 24$  at  $\lambda$  max 264  $m\mu$  at pH 7.0.  $[\alpha]_D^{26} + 58^\circ$  ( $c$ , 1 per cent in water).

ANALYTICAL. Calculated for  $C_{15}H_{24}N_2O_8$ : C, 49.99; H, 6.71; N, 7.77; mol. wt., 360.37. Calculated for  $C_{15}H_{26}N_2O_8$ : C, 49.72; H, 7.23; N, 7.73; mol. wt., 362.39. Found: C, 49.60; H, 7.05; N, 7.86. No ash.

Potentiometric titration in water indicated a molecular weight of 368 with  $pK_a$  values of 4.6, 7.5, and 8.8. The lower value was attributed to acetic acid. Actinobolin acetate is soluble in the lower alcohols and acetone while warm and is sparingly soluble in ethyl acetate.

*N-Acetylactinobolin.* Actinobolin acetate (0.20 Gm.) was treated with 2 ml. of warm acetic anhydride and allowed to stand overnight at room temperature. The crystalline mixture was filtered, washed with ethyl acetate, and dried in vacuo affording 0.16 Gm. of the N-acetyl derivative. White needles were obtained from absolute ethanol melting at 254 to 255 C. (dec.).  $a = 26$  at  $\lambda$  max 264  $m\mu$  in phosphate buffer pH 7.0;  $a = 27.2$  at  $\lambda$  max 262  $m\mu$  in 0.1 *N* hydrochloric acid;  $a = 44.5$  at  $\lambda$  max 288  $m\mu$  in 0.1 *N* sodium hydroxide.

ANALYTICAL. Calculated for  $C_{15}H_{22}N_2O_7$ : C, 52.62; H, 6.48; N, 8.18; mol. wt., 342.36. Calculated for  $C_{15}H_{24}N_2O_7$ : C, 52.32; H, 7.03; N, 8.14; mol. wt., 344.37. Found: C, 52.73; H, 6.52; N, 8.25; mol. wt. from titration, 338.

#### SUMMARY

A new broad-spectrum antibiotic, actinobolin, has been isolated by carbon adsorption and ion exchange techniques. Analyses indicate an empirical formula  $C_{13}H_{20-22}N_2O_6$ . The antibiotic is amphoteric and complexes iron (III) and aluminum readily. Other characteristic properties and several derivatives are described.

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#### BIBLIOGRAPHY

1. PITILLO, R. F.; FISHER, M. W.; McALPINE, R. J.; THOMPSON, P. E., AND EHRLICH, J.: Actinobolin, a new broad-spectrum antibiotic. Origin and biologic evaluation. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 497.
2. SUGIURA, K., AND REILLY, H. C.: The effect of actinobolin on a spectrum of tumors. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 522.
3. BURCHENAL, J. H.; HOLMBERG, E. A. D.; REILLY, H. C.; HEMPHILL, S., AND REPERT, J. A.: The effects of actinobolin on transplanted mouse leukemias. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 528.
4. TELLER, M. N.; MERKER, P. C.; PALM, J. E., AND WOOLLEY, G. W.: The effects of actinobolin on the subcutaneous growth of human tumors H.S.#1 and H.Ep.#3 in the rat. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 518.
5. MERKER, P. C., AND WOOLLEY, G. W.: The effects of actinobolin on human epidermoid carcinoma (H.Ep.#3) growing in the conditioned Swiss mouse. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 515.

# Actinobolin, a New Broad-Spectrum Antibiotic

## Pilot Plant Studies

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Actinobolin, a new broad-spectrum antibiotic produced by use of a *Streptomyces*, has been biologically characterized by Pittillo et al<sup>1</sup> and isolated and chemically characterized by Haskell and Bartz.<sup>2</sup> The purpose of this paper is to report the results of pilot plant studies on the fermentation and purification of actinobolin.

## FERMENTATION

Our initial fermentation studies were performed in 30 liter stirred jars. The medium used in these jars contained 1 per cent Cerelese, 1 per cent soybean oil meal, 0.5 per cent hog stomach residue (saline extracted), 0.167 per cent ammonium chloride, 0.5 per cent sodium chloride, and 0.1 per cent calcium carbonate. Prior to the addition of the calcium carbonate the pH was adjusted to 7.5 with sodium hydroxide. The ingredients were sterilized in the fermentors at 121 C. for 90 minutes. Foam was controlled by the automatic addition of Inedible Defoamer 51. The fermentations were incubated at 26 C., agitated at 200 revolutions/min. by a turbine impeller, and aerated with one volume of air per volume of medium per minute.

The pH of each sample was determined immediately after its withdrawal by means of a glass electrode. Actinobolin was assayed against *Sarcina lutea* PCI 1001W by the disc plate method. Carbohydrate was determined by the method of Somogyi.<sup>3</sup> Ammonia was determined by the method of Pierce and Haenisch.<sup>4</sup> Growth was determined by centrifugation of a whole beer sample in a 15 ml. graduated conical glass centrifuge tube for 12 minutes in an International clinical centrifuge at 2350 revolutions/min. and reported as per cent sediment.

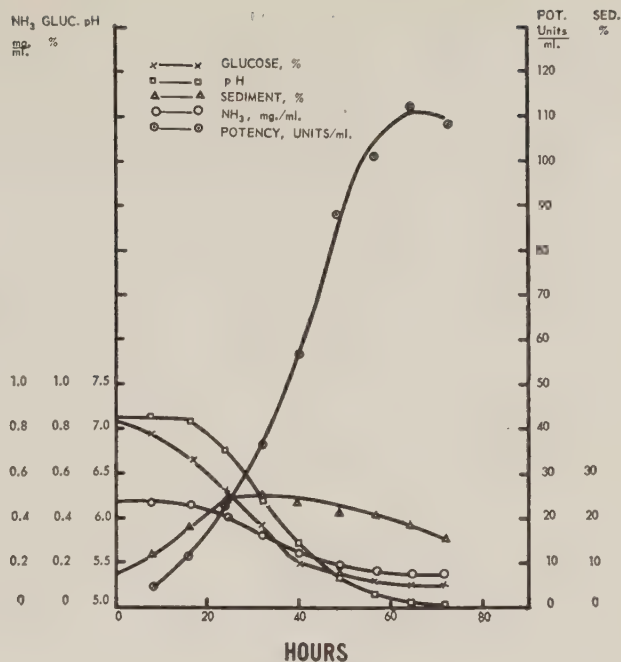
A typical biochemical fermentation pattern in stirred jars is presented in figure 1. It will be noted that peak antibiotic titers were obtained after 60 to 72 hours of incubation. Glucose and ammonia were utilized rapidly during the first 40 hours. Growth increased rapidly during the first 32 hours and thereafter levelled off or declined slightly. The pH decreased throughout the fermentations and reached a value of 5.0 to 5.1 after 72 hours of incubation.

Based on stirred jar studies, the fermentation was scaled up to 2000 gallon stainless steel fermentors. The medium for these fermentations consisted of 2 per cent Cerelese, 1 per cent soybean oil meal, 0.5 per cent Proto peptone 159, 0.167 per cent ammonium chloride, 0.5 per cent sodium chloride, 0.25 per cent calcium carbonate. Prior to the addition of the calcium carbonate the pH was adjusted to 7.5 with sodium hydroxide. The ingredients were sterilized batchwise in the fermentor for 60 minutes at 121 C. Foam was controlled by the addition of Inedible Defoamer 51. The fermentations were incubated at 26 C., agitated at 125 revolutions/min. by a dual turbine impeller, and aerated with 120 cubic feet of air per minute.

A typical biochemical fermentation pattern in the 2000 gallon fermentors is presented in figure 2. A comparison of figure 2 with figure 1 indicates a very close agreement with the exception of the pH curve. The additional calcium carbonate used in the larger fermentations resulted in the maintenance of a pH varying between the limits of 6.4 and 6.7.

Since the scale-up attempt was successful, the fermentation operating procedure

FIG. 1. A typical biochemical fermentation pattern in stirred jar fermentors is shown.



was standardized to the procedure just mentioned and a succession of essentially identical fermentations were run to provide broth for the isolation and purification of actinobolin.

#### FRACTIONATION

The fractionation procedure we adopted for the pilot plant scale purification of actinobolin sulfate is summarized.

*Darco G-60 Adsorption.* Filtration of the harvested beer (pH 4.0), was followed

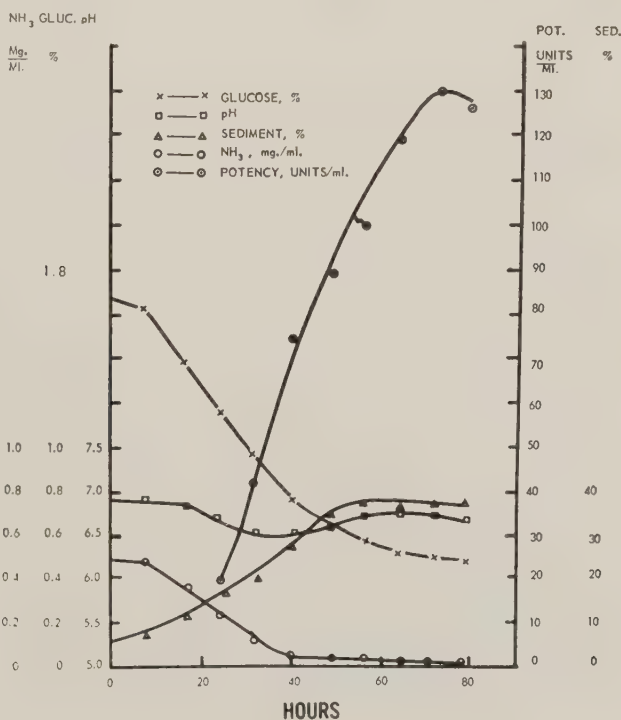


FIG. 2. A typical biochemical fermentation pattern in 2000 gallon fermentors is illustrated.

by batchwise adsorption of the activity on Darco G-60 (4 per cent w/v). The Darco G-60 was collected in a 30 inch plate and frame press, washed with water, and then eluted with 20 per cent acetone.

*Dowex 50 (Na<sup>+</sup>) Column.* The 20 per cent acetone eluate from above was percolated through a Dowex 50 (Na<sup>+</sup>) column. After applying the charge, the Dowex column was thoroughly washed with water and then eluted with aqueous 5 per cent sodium sulfate solution.

*Darco G-60 Chromatography.* After concentration and subsequent chilling to remove crystalline sodium sulfate, the remaining eluate was chromatographed on a Darco G-60 column. The column was washed with water until a negative test for sulfate ion was obtained, then eluted in fractions with 20 per cent aqueous acetone.

*Removal of Iron with Cupferron.* After combining the most active fractions and concentrating to a small volume, the iron was removed by extracting with cupferron in 50:50 *n*-butanol-chloroform solution. This procedure removed iron from the iron-actinobolin complex liberating free actinobolin sulfate which was further purified by crystallization from ethanol-water at 45-50 C.

This procedure differs from that described by Haskell and Bartz<sup>2</sup> in that we substituted a Dowex 50 (Na<sup>+</sup>) column for the Decalso column they described. This change enabled us more easily to prepare the antibiotic in higher yields on a pilot plant scale without metal contamination.

#### EXPERIMENTAL

*Darco G-60 Adsorption.* The harvest beer (4750 gallons) was adjusted to pH 4.0 and filtered through a 38 inch plate and frame press in two portions. To the clarified filtered beer ( $2.24 \times 10^9$  units) were added 1530 pounds of Darco G-60 and after agitation for 30 minutes the mixture was filtered in a 30 inch plate and frame press, again, in two portions. After washing with water, the two carbon cakes were eluted with a total of 6000 gallons of 20 per cent acetone. The eluate ( $1.55 \times 10^9$  units, 69 per cent) was then used as such in the next step. A dried aliquot of the eluate usually assayed 25 to 30 per cent pure at this stage.

*Dowex 50 (Na<sup>+</sup>) Ion Exchange Adsorption.* The acetone eluate ( $1.55 \times 10^9$  units) was ion exchanged on 250 liters of Dowex 50 (Na<sup>+</sup>) contained in an 18 inch diameter, 6 foot tall column at a flow rate of 150 gallons/hour. No leakage of antibiotic activity was observed. After washing the column thoroughly with water, it was eluted with aqueous 5 per cent sodium sulfate solution. The progress of the elution was followed easily by testing with acidic ferric chloride solution. When the test became negative, the elution was stopped. The eluate (1500 gallons) assaying  $1.42 \times 10^9$  units (92 per cent) was concentrated under vacuum at <35 C. to 300 gallons. This concentrate was cooled in a conical glycol-cooled tank to 0 C. with constant agitation. The crystals of sodium sulfate which formed were removed by filtration and washed with water to give a final solution of 800 gallons. This solution was concentrated to 125 gallons, recooled, and refiltered. The final volume, after concentration to a suitable volume for application to carbon columns, was 85 gallons assaying  $1.23 \times 10^9$  units (86.6 per cent of the sodium sulfate eluate). Additional material could be recovered by increased washing of the sodium sulfate crystals. Six columns (four containing 36 Kg. Darco and 36 Kg. Celite each, and two containing 40 Kg. Darco G-60 and Celite 545 each, in 12 inch diameter glass lined columns) were needed for the purification of this entire lot.

*Darco G-60 Chromatography.* The progress of only one column will be described, since the others followed the same pattern. Seventeen gallons (64.35 liters) of con-

TABLE I  
Summary of the Chromatography

Sample	Volume, liters	ou/ml.	Total ou at $\lambda$ 264 $m\mu$	Per cent of S.M.	$E_{1\text{ cm.}}^{1\%}$ at $\lambda$ 264 $m\mu$ (0.1 M phosphate buffer, pH 7.0)
S.M.*	64.35	1460	$93.95 \times 10^6$		
1	10	105	$1.05 \times 10^6$	1.12	110
2	10	380	$3.80 \times 10^6$	4.05	208
3	10	490	$4.90 \times 10^6$	5.21	222
4	10	570	$5.70 \times 10^6$	6.07	224
5	10	540	$5.40 \times 10^6$	5.75	228
6	10	470	$4.70 \times 10^6$	5.00	235
7	10	450	$4.50 \times 10^6$	4.78	232
8	10	420	$4.20 \times 10^6$	4.48	226
9	302.8	160	$48.45 \times 10^6$	51.75	237
10	196.8	25.3	$4.98 \times 10^6$	5.30	238
			$87.68 \times 10^6$	93.33	

\* S.M. = Starting material.

centrated sodium sulfate eluate was applied to a 12 inch diameter, 12 foot tall column which had previously been packed with 40 Kg. Darco G-60 and 40 Kg. Celite 545. The column was run under a pressure of 30 pounds/sq. in. The charge, 64.35 liters, containing  $93.95 \times 10^6$  optical units,\* was followed by water until the percolate from the column gave a negative test for sulfate ion. The column was then eluted with 20 per cent acetone. Since the activity usually emerged immediately after the passage of one holdup volume of 20 per cent acetone, the eluate was checked carefully until a positive test with acidic ferric chloride was obtained. Then, eight 10 liter fractions were taken, followed by two larger fractions, 302.8 liters and 196.8 liters. Since the microbial activity and the ultraviolet activity correlate so closely at this point, the progress of the purification was followed by checking the ultraviolet absorption at  $\lambda$  264  $m\mu$ . After  $E_{1\text{ cm.}}^{1\%}$  values were taken on lyophilized aliquots the better fractions were combined with all similar fractions from the other five columns for further work-up. The chromatography is summarized in table I.

Since the  $E_{1\text{ cm.}}^{1\%}$  of the pure material is 253 at  $\lambda$  264  $m\mu$  (pH 7.0, 0.1 M phosphate buffer) it is apparent that the eluate fractions are almost pure.

Fractions 3 to 10 from this column and similar fractions from the other five columns were combined and concentrated to 29 gallons ( $0.9 \times 10^9$  units) which was then treated to remove iron.

**Iron Removal with Cupferron.** To 45 gallons of a 50:50 mixture of *n*-butanol and chloroform was added 500 Gm. of cupferron (N-nitrosophenyl hydroxylamine ammonium salt); this solution was divided into three 15 gallon aliquots, which were used repeatedly to extract the 29 gallons of active eluate concentrate derived from the carbon columns. The first 15 gallon extract was intensely red in color, and the two succeeding extracts diminished in color until very little color was obtained in the third extract. The aqueous phase remaining after the third extraction was extracted four times with 15 gallon portions of 50:50 *n*-butanol-chloroform to remove any residual cupferron. The aqueous phase was then treated with sulfuric acid washed Darco G-60 (6 Kg.) to remove pyrogen (yield 87.5 per cent) and then

\* An optical unit (ou) is that amount of actinobolin contained in 1 ml. of solution which exhibits an optical density of 1 at  $\lambda$  264  $m\mu$ . The number of ou in a given sample is obtained by multiplying the optical density at the given wavelength by the dilution required to give that optical density.

lyophilized to give 10,576 Gm. of partially purified actinobolin sulfate ( $E_{1\text{cm.}}^{1\%} = 220$ ). This was combined with 751 Gm. of similar material from another run and crystallized from a water-ethanol mixture. The product (11,327 Gm.) was dissolved in 14.2 liters of water (1.25 ml./Gm.) and stirred vigorously with warming to 45 to 50 C. When the product was completely dissolved, 45.3 liters of anhydrous ethanol (4 ml./Gm.) was added with constant stirring while maintaining the mixture at 45 to 50 C. Crystals started to separate after a few minutes. The heating was discontinued after two hours and the mixture was allowed to cool to room temperature overnight. The mixture was filtered and vacuum dried to give 8387 Gm. of crystalline actinobolin sulfate:  $E_{1\text{cm.}}^{1\%} = 238$  at  $\lambda$  max 264 m $\mu$  (0.1 M phosphate buffer, pH 7.0),  $[\alpha]_D^{26} = +55^\circ$  (c, 1 per cent in phosphate buffer, pH 7.0), microbiological assay = 80 units/mg. Crystalline actinobolin sulfate produced by this method was identical in all respects to the crystalline antibiotic produced by Haskell and Bartz.<sup>2</sup>

#### SUMMARY

The pilot plant fermentation and purification of a new broad-spectrum antibiotic, actinobolin sulfate, is described. Owing to the powerful chelating properties of this antibiotic for both iron and aluminum, a method of purification was devised that enabled us to prepare more easily the metal-free antibiotic on a pilot plant scale. This was accomplished by a series of purification steps which were as follows: (1) Adsorption on Darco G-60 and elution with 20 per cent acetone; (2) ion exchange adsorption on Dowex 50 ( $\text{Na}^+$ ) and elution with aqueous 5 per cent sodium sulfate; (3) chromatography on columns composed of a 50:50 mixture of Darco G-60 and Celite 545, and elution with 20 per cent aqueous acetone; (4) removal of chelated iron from the best Darco G-60 fractions by extraction with cupferron in 50:50 *n*-butanol-chloroform; (5) crystallization from ethanol-water at 45 to 50 C.

Crystalline actinobolin sulfate so prepared was identical in all respects with the actinobolin sulfate prepared by Haskell and Bartz.<sup>2</sup>

#### ACKNOWLEDGMENTS

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#### BIBLIOGRAPHY

1. PITILLO, R. F.; FISHER, M. W.; McALPINE, R. J.; THOMPSON, P. E., AND EHRLICH, J.: Actinobolin, a new broad-spectrum antibiotic. Origin and biologic evaluation. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 497.
2. HASKELL, T., AND BARTZ, Q. R.: Actinobolin, a new broad-spectrum antibiotic. Isolation and characterization. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 505.
3. HODGE, J. E., AND DAVIS, H. A.: Selected methods for determining reducing sugars. *Northern Regional Research Laboratories, Peoria, Illinois, Apr., 1952*, p. 13.
4. PIERCE, W. C., AND HAENISCH, E. L.: *Quantitative Analysis*, Ed. 2, New York, John Wiley & Sons, 1946, p. 127.

# The Effects of Actinobolin on Human Epidermoid Carcinoma (H.Ep.#3) Growing in the Conditioned Swiss Mouse

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Potential anticancer agents have been tested against human epidermoid carcinoma (H.Ep.#3) growing in the embryonated egg<sup>1,2</sup> and in the conditioned rat<sup>3,4</sup> and hamster.<sup>5</sup> It has been reported that H.Ep.#3 can be grown in conditioned mice.<sup>6,7</sup> In order to establish the usefulness of the mouse H.Ep.#3 system, a series of chemical agents, known antibiotics, and crude culture filtrates have been selected for testing. Because the new antibiotic, actinobolin,<sup>8</sup> displayed moderate inhibitory activity against the growth of sarcoma 180,<sup>9</sup> the effects of this agent on the growth of H.Ep.#3 in the mouse has been studied.

## METHOD

Female Swiss Webster mice (Taconic Farms) weighing 18 to 22 Gm. were used as hosts. Because adequate amounts of tumor tissue are needed for setting up chemotherapy groups, it is simpler to use large rat-grown rather than small mouse-grown H.Ep.#3 as a source of tumor for transplantation. Therefore, 7 to 14 day old H.Ep.#3 grown intramuscularly in the conditioned rat as per the method of Toolan<sup>10</sup> was prepared as a 50 per cent (w/v) suspension by mincing the tissue with a pair of sterile scalpels. The diluent consisted of sterile normal saline fortified with potassium penicillin G (1000 units/ml.) and streptomycin sulfate (2 mg./ml.). One half ml. of tumor suspension was injected through a 16 gauge needle into the right thigh muscle of mice roentgen-ray irradiated three to five days previous to transplantation (100 r, total body). Immediately after transplantation, each mouse received cortisone acetate at a dose of 1.5 mg./mouse; three additional doses of cortisone at 1.0 mg./mouse were given on alternate days after transplantation. Nine days after transplantation, the animals were sacrificed, and net tumor weight was determined by subtracting the weight of the opposite, uninjected leg from the weight of the tumor-bearing leg. The transplant and conditioning procedures are essentially those of Toolan<sup>6,10</sup> as modified by Gallily and Woolley.<sup>7</sup>

Actinobolin was prepared in normal saline in a concentration such that the dose could be administered in a total volume of 1.0 ml. (0.5 ml. twice daily). Therapy was started 24 hours after transplantation and continued for seven days (Sundays excepted). Antitumor activity was evaluated by comparing the average tumor weights from 3 treated and 3 to 6 control mice. Control mice received intraperitoneal or oral doses of equivalent volumes of normal saline. All mice were fed Purina laboratory chow; drinking water was supplemented with oxytetracycline (animal formula) at an approximate concentration of 7 mg./ml.

## RESULTS AND CONCLUSION

Prior to chemotherapy trials, preliminary analysis on 132 tumor-bearing mice revealed that the average tumor weight was 2.3 Gm., the average range in tumor

TABLE I  
Effect of Actinobolin on the Growth of Intramuscular Transplants of  
H.Ep.#3 in the Conditioned Swiss Mouse

Dose, mg./Kg. X7	Route	Host body weight change, Gm.		Mortality		Av. tumor wt., Gm.		% inhibition
		Treated	Control	Treated	Control	Treated	Control	
2000	Intraperitoneal			2/3	0/3			
2000	Intraperitoneal			2/3 <sup>+</sup>	0/3	1.2	3.3	64
2000	Oral			2/3	1/6			
1000	Intraperitoneal	—3	—2	0/3	0/3	1.3	1.3	0
1000	Intraperitoneal	—3	+2	0/3	0/3	1.2	1.8	33
1000	Intraperitoneal	0	+2	1/3	1/6	1.7	3.3	49
1000	Oral	0	+1	0/3	0/6	1.6	2.1	24
1000	Oral	+1	+2	0/3	1/6	2.7	3.3	18

+ One animal died on day of autopsy, tumor weight used in evaluation.

weight for groups of three tumors was 1.2 Gm., and the standard deviation (based on the average range for groups of three) was 0.69 Gm. Mortality at the end of the nine day period of growth, for 153 tumor transplanted mice was 4.6 per cent; tumors grew in 100 per cent of the transplanted mice.

Data in table I indicate that actinobolin was lethal to H.Ep.#3 tumor transplanted mice at a dose of 2000 mg./Kg. Several animals, given this dose orally, died as early as four days after the start of therapy. In one experiment, at this dose, two tumors were available for averaging because one of the mice having lost 7 Gm. in body weight, died on the day of autopsy. The 64 per cent inhibition obtained on this test must therefore be evaluated in relation to the lethality of the drug.

At a dose of 1000 mg./Kg., actinobolin was not lethal, but there were indications of slight depressing effects on host body weight. For three experiments using the intraperitoneal route of administration, the antitumor effect was 42 per cent based on the averages obtained by pooling individual tumor weights from all three experiments. The effects for the individual experiments were 0, 33, and 49 per cent inhibition. The average inhibitory effect for the oral route was less than 25 per cent.

Under the experimental conditions of the test and on the basis of these data, actinobolin, at non-lethal doses, does not significantly inhibit human epidermoid carcinoma (H.Ep.#3) growing intramuscularly in the conditioned Swiss mouse. In studies with H.Ep.#3 growing in the hamster cheek pouch<sup>5</sup> and in the embryonated egg<sup>11</sup> the antibiotic has not displayed significant antitumor activity. In other studies, actinobolin has been found to inhibit H.Ep.#3 growing subcutaneously in the conditioned rat.<sup>12</sup>

Although data on the mouse H.Ep.#3 system agree in general with findings in the hamster and embryonated egg H.Ep.#3 systems, the possibility must be kept in mind that the conditioning agents may influence the action of actinobolin.

#### SUMMARY

1. Actinobolin, at 2000 mg./Kg. given intraperitoneally or orally, is lethal to roentgen-ray irradiated and cortisone-treated Swiss mice transplanted with human epidermoid carcinoma (H.Ep.#3).

2. Actinobolin, at 1000 mg./Kg., administered orally or intraperitoneally, does not significantly inhibit human epidermoid carcinoma (H.Ep.#3) growing intramuscularly in the roentgen-ray irradiated and cortisone-treated Swiss mouse.

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# BIBLIOGRAPHY

1. DAGG, C. P.; KARNOFSKY, D. A.; TOOLAN, H. W., AND RODDY, J.: Serial passage of human tumors in chick embryo: Growth inhibition by nitrogen mustard, *Proc. Soc. Exper. Biol. & Med.* 87:223-227, 1954.
2. HARRIS, J. J.: Differences in the behavior of two human tumors grown on the choriollantois of the chick embryo, *Cancer* 9:756-759, 1956.
3. MERKER, P. C.; TELLER, M. N.; PALM, J. E., AND WOOLLEY, G. W.: Effect of actinomycin D on two human tumors growing in conditioned rats, *Antib. & Chemo.* 7:247-250, 1957.
4. TELLER, M. N.; PALM, J. E.; MERKER, P. C.; HARRIS, J. J., AND WOOLLEY, G. W.: The use of transplantable human tumors in experimental chemotherapy, *Cancer Research* 18:522-526, 1958.
5. PALM, J. E.: Personal communication.
6. TOOLAN, H. W.: Growth of transplantable human tumors in conditioned mice, *Transplantation Bull.* 4:160, 1957.
7. GALLILY, R., AND WOOLLEY, G. W.: The human tumor in the mouse, *Ann. New York Acad. Sc.* 1958. In press.
8. PITTILLO, R. F.; FISHER, M. W.; MCALPINE, R. J.; THOMPSON, P. E., AND EHRLICH, J.: Actinobolin, a new broad-spectrum antibiotic. Origin and biologic evaluation. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 497.
9. REILLY, H. C.: Personal communication.
10. TOOLAN, H. W.: Transplantable human neoplasms maintained in cortisone-treated laboratory animals: H.S.#1; H.Ep.#2; H.Ep.#3; H.Emb. Rh.#1, *Cancer Research* 14:660-666, 1954.
11. HARRIS, J. J.: Personal communication.
12. TELLER, M. N.; MERKER, P. C.; PALM, J. E., AND WOOLLEY, G. W.: The effects of actinobolin on the growth of human tumors H.S.#1 and H.Ep.#3 in the rat. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 518.

# The Effects of Actinobolin on the Growth of Human Tumors H.S.#1 and H.Ep.#3 in the Rat

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Potential anticancer compounds of various chemical compositions and of diverse origins have been tested in the human tumor rat host experimental chemotherapy systems.<sup>1-5</sup> These have included a number of microbiological culture filtrates in various stages of purification. Of the compounds tested, only a small number were found to inhibit markedly the growth of the experimental human tumors; these included several of microbiological origin, indicating that microbiological products may prove to be a fertile source of antitumor drugs.

Actinobolin, a new antibiotic,<sup>6</sup> reported as having antitumor activity against sarcoma 180 in the Swiss mouse,<sup>7</sup> was recommended by Reilly for tests in the human tumor experimental chemotherapy systems. This paper deals with the effects of actinobolin on the subcutaneous growth of the human sarcoma H.S.#1 and the human epidermoid carcinoma H.Ep.#3 in the conditioned rat.

## MATERIALS AND METHODS

The test procedure has been described in detail elsewhere.<sup>1</sup> Briefly, young female albino rats were roentgen-ray irradiated with a single total body dose of 150 r one to three days prior to transplantation of tumor tissue. The transplantable human tumors used were the sarcoma H.S.#1 and the epidermoid carcinoma H.Ep.#3, descriptions of which have been published by Toolan.<sup>8</sup> For chemotherapy trials, 0.5 ml. of a 70 per cent suspension (w/v) was injected subcutaneously in the right flank of the irradiated rat. Immediately after transplantation of the tissue, the rats were injected subcutaneously near the nape of the neck with cortisone acetate at a dose of 60 mg./Kg. of body weight. Rats bearing H.S.#1 tumors were injected again with cortisone on alternate days for a total of four doses; those bearing H.Ep.#3 tumors were injected at three to four day intervals for a total of three doses.

Drug therapy was begun 24 hours after tumor transplantation and was administered intraperitoneally twice daily (except Sundays) unless otherwise indicated. Actinobolin was dissolved in physiological saline in concentrations such that the dose was administered in 0.5 to 0.6 ml. Control animals received equal amounts of physiological saline.

Rats bearing H.S.#1 were sacrificed on the eleventh to twelfth day, and those bearing H.Ep.#3 on the ninth to eleventh day post-transplantation, 24 hours after administration of the last dose. The tumors were removed, opened, and all liquid and necrotic material removed before weighing. The difference between the average tumor weights of the treated and control groups was then calculated as percentage inhibition.

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TABLE I  
*The Effects of Intraperitoneal Injections of Actinobolin  
on Human Sarcoma H.S.#1 in the Rat*

Dose, mg./Kg./day	Toxicity						% inhibition
	Av. weight change, Gm.		Mortality		Av. tumor weight, Gm.		
	Treated	Control	Treated	Control	Treated	Control	
1000*	+ 5	+22	2/6	0/6	1.1	4.4	75
500	+ 8	+ 6	0/6	3/6	0.7	3.2	78
	+ 1	+ 1	0/6	0/6	1.7	4.8	65
250	+23	+22	0/6	0/6	2.5	4.4	43
125	+15	+11	0/6	1/6	3.2	1.7	0
1000†	+ 3	+12	1/6	0/6	7.2	8.2	12

\* Highly toxic, animals in poor condition.

† First injection of compound on fourth day post-transplantation.

## RESULTS

The effects of actinobolin on the growth of H.S.#1 and H.Ep.#3 in the rat are given in tables I and II, respectively.

H.S.#1 was inhibited 75 per cent at a dose of 1000 mg./Kg. (table I), but only one measurable tumor was found in the 4 survivors, no tumor growth being seen in the other 3. There were no "negatives" (absence of tumor growth) in the control group. Toxic effects were apparent by the poor condition of the animals at the end of the experiment. Although the toxic symptoms of diarrhea and ruffled hair were not reflected by the average weight change of the surviving rats (+5 Gm.), 2 of the 6 rats in the treated group died. At the lower dose of 500 mg./Kg., the inhibitions were 78 and 65 per cent, decreasing to 43 per cent at 250 mg./Kg. There was no inhibition of H.S.#1 at 125 mg./Kg., nor in the test at 1000 mg./Kg. when the first injection of actinobolin was given on the fourth day post-tumor transplantation.

Actinobolin at 1000 mg./Kg. was not so toxic to rats bearing H.Ep.#3 as to those bearing H.S.#1 tumors. Inhibitions of 75 and 77 per cent were obtained at this dose with little or no toxicity (table II). At 500 mg./Kg., H.Ep.#3 was inhibited 57 and 54 per cent in two tests, but no effect was seen at a dose of 250 mg./Kg. When tested at 1000 mg./Kg. against established tumors (initial dose four days post-transplantation), inhibitions of H.Ep.#3 tumor growth were 61, 0, and 23 per cent in three tests. In the trial resulting in 61 per cent inhibition, the average

TABLE II  
*The Effects of Actinobolin on Human Carcinoma H.Ep.#3 Growing  
Subcutaneously in the Rat*

Dose, mg./Kg./day	Route	Toxicity				Av. tumor weight, Gm.		% inhibition
		Av. weight change, Gm.		Mortality		Treated	Control	
		Treated	Control	Treated	Control			
1000	Intraperitoneal	+ 9	+11	0/5	0/5	1.0	4.1	75
		— 3	+ 6	0/3	1/3	1.3	5.7	77
500	Intraperitoneal	+ 4	+ 5	0/6	1/6	1.8	4.2	57
		+12	— 4	0/6	1/6	1.6	3.5	54
250	Intraperitoneal	+21	+15	1/6	0/6	5.8	3.8	0
1000	Oral	+12	+11	0/6	0/6	2.1	2.8	25
1000*	Intraperitoneal	+ 8	+23	0/6	0/6	3.5	8.9	61
		+14	+21	0/6	0/6	7.0	1.8	0
		+ 8	+22	1/6	0/6	1.7	2.2	23

\* First injection of compound on fourth day post-transplantation.

tumor growth in the control group was unusually large. Slight inhibition occurred when actinobolin was administered orally at 1000 mg./Kg.

#### DISCUSSION AND CONCLUSIONS

The effects of actinobolin on the transplanted human tumors H.S.#1 and/or H.Ep.#3 growing subcutaneously in the conditioned rat were determined by three types of tests: intraperitoneal administration initiated 24 hours after tumor transplantation, intraperitoneal administration initiated on the fourth day post-transplantation, and oral administration initiated 24 hours post-transplantation. Good inhibitory effects against both tumors occurred when the compound was administered intraperitoneally beginning 24 hours post-transplantation. Little or no inhibition was found when actinobolin was administered either orally (H.Ep.#3), or intraperitoneally beginning 24 hours after tumor transplantation (H.S.#1, H.Ep.#3). Although a 61 per cent inhibition of H.Ep.#3 occurred in one of the "post-4-day" tests, the average weight of the control tumors was inordinately large, casting doubt on the magnitude of the inhibitory effect. The lack of significant inhibition of established tumors was substantiated in the two additional tests. It appears, then, that actinobolin may inhibit the growth of young human tumor tissue, but has little effect on established tumors. It should be noted, however, that the sarcoma H.S.#1 and the carcinoma H.Ep.#3 both were inhibited by actinobolin.

The dose of 1000 mg./Kg., when injected 24 hours after tumor transplantation, was found to be more toxic to H.S.#1 bearing rats than to those bearing H.Ep.#3 tumors, despite the similarity in conditioning procedures. This phenomenon had been noted previously with other compounds<sup>1,2</sup> and was found not to be due to the additional dose of cortisone for the H.S.#1 bearing rats.

#### SUMMARY

Actinobolin, when administered intraperitoneally beginning 24 hours after tumor transplantation, had moderate to good inhibitory effects on the subcutaneous growth of the two transplantable human neoplasms, H.S.#1 and H.Ep.#3, in the conditioned rat. These effects were obtained at tolerated doses. Little or no effect occurred against established tumors (initial dose on the fourth day post-transplantation), or when the compound was administered orally beginning 24 hours after tumor transplantation.

#### ACKNOWLEDGMENTS

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#### BIBLIOGRAPHY

1. TELLER, M. N.; PALM, J. E.; MERKER, P. C.; HARRIS, J. J., AND WOOLLEY, G. W.: The use of transplantable human tumors in experimental chemotherapy, *Cancer Research* 18:522-526, 1958.

2. TELLER, M. N.; MERKER, P. C.; PALM, J. E., AND WOOLLEY, G. W.: The human tumor in cancer chemotherapy in the conditioned rat, *Ann. New York Acad. Sc.* 1958. In press.
3. MERKER, P. C.; PALM, J. E., AND TELLER, M. N.: The effects of a series of chemical agents on transplantable human tumors growing in the rat, *Proc. Am. A. Cancer Research* 2:133, 1956.
4. TELLER, M. N., AND PALM, J. E.: Anti-tumor effects of several antibiotics on transplantable human tumors in the conditioned rat, *Proc. Am. A. Cancer Research* 2:255, 1957.
5. MERKER, P. C.; TELLER, M. N.; PALM, J. E., AND WOOLLEY, G. W.: Effect of actinomycin D on two human tumors growing in conditioned rats, *Antib. & Chemo.* 7:247-250, 1957.
6. PITILLO, R. F.; FISHER, M. W.; MCALPINE, R. J.; THOMPSON, P. E., AND EHRLICH, J.: Actinobolin, a new broad-spectrum antibiotic. Origin and biologic evaluation. *Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 497.
7. REILLY, H. C.: Personal communication.
8. TOOLAN, H. W.: Transplantable human neoplasms maintained in cortisone-treated laboratory animals: H.S.#1; H.Ep.#2; H.Ep.#3; H.Emb. Rh#1, *Cancer Research* 14:660-666, 1954.

# The Effect of Actinobolin on a Spectrum of Tumors

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One of the most actively investigated areas of cancer chemotherapy concerns the study of products of microorganisms. Experimental evidence obtained within the last few years has indicated that certain antibiotics have strong antitumor effects on certain animal neoplasm.<sup>7,15</sup> Actinomycins C, D, and J<sup>1,3-6,8,18,19</sup> and mitomycin C<sup>9,10,17</sup> have shown promising effects against various types of human cancer.

This report consists of observations on the effect of actinobolin, a new antibiotic isolated from broth cultures of a *Streptomyces*, on the growth of 12 solid tumors of the mouse, three solid tumors of the rat, two ascites tumors of the mouse, and one mouse virus leukemia.

## MATERIALS AND METHODS

The tumors used in the present study are listed in table I as follows: Crocker sarcoma 180, sarcoma 180 ascites tumor, sarcoma T 241, Ehrlich carcinoma, Ehrlich ascites carcinoma, adenocarcinoma E 0771, Miyono adenocarcinoma, carcinoma 1025, Lewis bladder carcinoma, Ridgway osteogenic sarcoma, Mecca lymphosarcoma, Gardner lymphosarcoma, Harding-Passey melanoma, glioma 26, and Friend virus leukemia in mice, Walker carcinosarcoma 256, Jensen sarcoma, and Murphy-Sturm lymphosarcoma in rats.

The methods employed in the chemotherapy studies were as follows: for the solid tumors,<sup>12</sup> subcutaneous implantations of tumors (small pieces of tumor, each weighing approximately 6 mg.) into healthy young animals (18 to 22 Gm. mice; 80 to 100 Gm. rats) were carried out by the usual trocar method with a single implant into the right axillary region. In every set of experiments, tumor-bearing animals were divided into two groups, one of controls and the other for treatment with antibiotics. The progress of the tumors in the animals was recorded graphically by measuring them in two diameters with calipers at weekly intervals for four weeks after tumor transplantation.

In the case of ascites tumors<sup>11</sup> the intraperitoneal injection of 0.1 ml. of the fluid containing about 1 million cancer cells was made into each mouse in the inguinal region from a ¼ ml. syringe fitted with a 25 gauge needle. These mice regularly develop large amounts of a milky ascites in 7 to 14 days and die in 10 to 20 days.

In the case of Friend virus leukemia<sup>14</sup> the intraperitoneal injection of 0.2 ml. of a 10 per cent saline homogenate of leukemic spleens was made into each mouse in the inguinal region. This transplanted leukemia kills mice in two to four months with marked enlargement of liver and spleen. The effects of the antibiotics have been evaluated by comparing weights of spleens in treated and untreated infected mice three weeks after intraperitoneal injection of the leukemic spleen homogenate. At this time spleens of leukemic mice weigh about 2.0 Gm., while that of normal female Swiss albino mice (about 8 weeks old) weighs about 0.2 Gm. For the convenience of routine screening purposes, the three weeks' observation period instead

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TABLE I

*Effect of Actinobolin, 1000 mg./Kg./day, on Various Mouse and Rat Tumors*

Tumor	No. of deaths		Av. wt. change, Gm., treated/controls 1st week	Results of treatment*		Remarks
	1st week	2nd week		1st week	2nd week	
Sarcoma 180	0/10	1/10	+0.7/+2.9	±	—	
Sarcoma 180 (ascitic)	0/10	1/10	—1.0/+5.7	+++	+++	+++ at 3 weeks
Sarcoma T 241	0/5	0/5	—0.9/+0.1	—	—	
Ehrlich carcinoma	0/5	0/5	+0.1/+2.1	±	±	
Ehrlich carcinoma (ascitic)	0/10	1/10	—0.7/+6.0	++	+++	+++ at 3 weeks
Adenocarcinoma E 0771	1/10	3/10	—2.1/+0.5	±	+	± at 3 weeks
Miyono adenocarcinoma	0/5	0/5	—2.2/+3.3	+	±	
Carcinoma 1025	0/10	0/10	—3.3/+0.1	±	++	++ at 3 weeks
Lewis bladder carcinoma	0/5	0/5	—2.6/—0.5	—	—	
Ridgway osteogenic sarcoma	0/5	1/5	—4.5/+2.1	—	—	
Mecca lymphosarcoma	0/10	0/10	—3.3/+1.1	+	—	
Gardner lymphosarcoma	0/5	0/5	—0.1/+0.1	±	—	
Harding-Passey melanoma	0/5	0/5	—1.0/+0.3	—	—	
Glioma 26	0/10	2/10	—2.2/+1.0	±	++	+ at 3 weeks
Friend virus leukemia	0/10	0/10	—0.7/+2.7		± (3w)	62†
Walker carcinosarcoma 256‡	0/5	0/5	+22/+27	++	+	
Walker carcinosarcoma 256	0/5	1/5	—1/+20	±	++	+ at 3 weeks
Jensen sarcoma‡	0/5	0/5	+27/+32	++	±	
Jensen sarcoma	0/5	0/5	+8/+29	++	±	
Murphy-Sturm lymphosarcoma	0/5	0/5	+14/16	++	±	

\* — indicates no effect; ± indicates slight inhibition; + indicates moderate inhibition; ++ indicates marked inhibition; +++ indicates complete inhibition.

† Per cent of spleen weight, treated/controls.

‡ Dose: 500 mg./Kg./day.

of the survival time was chosen. The animals were maintained on a standard pellet diet (Purina laboratory chow) and water ad libitum.

Intraperitoneal injection of the antibiotics at maximum tolerated doses was begun 24 hours after inoculation with tumor material and was continued once daily for seven days.

The degree of inhibition of the growth of the solid tumors was graded according to the following scheme (fig. 1): —, no effect (tumor growth to three quarters or more of the diameter of the controls); ±, slight inhibition (tumor growth from one half to three quarters of the diameter of the controls); +, moderate inhibition (tumor growth from one fourth to one half of the diameter of the controls); ++, marked inhibition (failure to grow or growth to approximately one fourth of the average diameter of the controls); +++, complete inhibition or destruction of tumors.

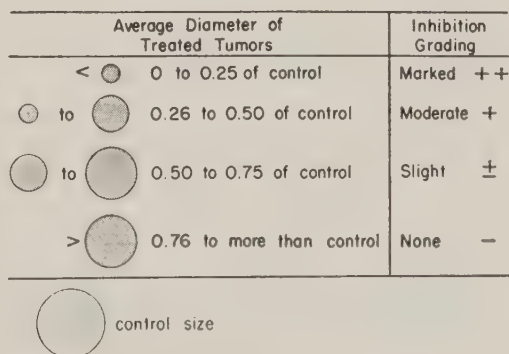








FIG. 1. The degree of inhibition of solid tumor growth is shown.



FIG. 2. The degree of inhibition of ascites tumor growth is illustrated: — indicates no effect;  $\pm$  indicates slight inhibition; + indicates moderate inhibition; ++ indicates marked inhibition.

The degree of inhibition of ascites tumor growth was graded according to the following scheme (fig. 2): —, no effect (marked abdominal distention; the fluid volume increases to three fourths or more of the controls, 10 to 25 ml. ascites);  $\pm$ , slight inhibition (moderate abdominal distention; the fluid volume increases to about one half the volume of the controls, 5 to 9 ml. ascites); +, moderate inhibition (slight abdominal distention; the fluid volume increases to about one fourth the volume of the controls, 1 to 4 ml. ascites); ++, marked inhibition (no abdominal distention; no gross ascites); +++, indicates complete inhibition or destruction of ascites.

The degree of inhibition of virus leukemia was graded according to the following scheme (fig. 3): —, no effect (spleen weight from three quarters or more of the average spleen weight of the leukemic spleens, controls);  $\pm$ , slight inhibition (spleen weight from one half to three quarters of the average spleen weight of the controls); +, moderate inhibition (spleen weight from one fourth to one half of the average spleen weight of the controls); ++, marked inhibition (spleen weight approximately one fifth of the average spleen weight of the controls); +++, complete

Average spleen weight of treated animals	Inhibition grading
 > 0.76 to more than control	None —
 to  0.50 to 0.75 of control	Slight $\pm$
 to  0.26 to 0.50 of control	Moderate +
 < 0 to 0.25 of control	Marked ++



 Control size

FIG. 3. The degree of inhibition of virus leukemia is given.

TABLE II  
*Effect of Antibiotics on Mouse, Rat, and Hamster Tumors\**

Tumor	Actinobolin, 1000 mg./Kg./day	Actinomycin D, 0.025 mg./Kg./day	Kanamycin, 500 mg./Kg./day	Mitomycin C, 2 mg./Kg./day
Sarcoma 180	±	—	—	+
Sarcoma 180 (ascitic)	+++	+++	—	+++
Sarcoma T 241	—	—	—	±
Sarcoma MA 387	—	—	—	±
Ehrlich carcinoma	±	—	—	±
Ehrlich carcinoma (ascitic)	+++	+++	—	+++
Bashford carcinoma 63	—	—	—	+
Adenocarcinoma E 0771	+	+	±	++
Miyono adenocarcinoma	±	—	—	++
Carcinoma 1025	++	++	—	+++
Lewis bladder carcinoma	—	—	—	±
Lewis lung carcinoma	—	—	—	+
Wagner osteogenic sarcoma	—	—	—	++
Ridgway osteogenic sarcoma	—	+++	—	+++
Mecca lymphosarcoma	—	—	—	±
Gardner lymphosarcoma	—	—	—	+
Harding-Passey melanoma	—	±	—	++
Glioma 26	++	—	—	++
Friend virus leukemia	±	—	—	+++
Flexner-Jobling carcinoma	—	—	—	+++
Walker carcinosarcoma 256	++	—	—	+++
Jensen sarcoma	±	±	—	+++
Murphy-Sturm lympho- sarcoma	±	—	—	++
Crabb hamster sarcoma	—	—	—	++

\* For explanation of grading of effects see footnote to table I.

inhibition (spleen weight less than one tenth of the average spleen weight of the controls).

## RESULTS

Results with actinobolin are recorded in table I. Each test group consisted of 5 animals. Experiments were repeated in many cases; the data presented are averages of the results of multiple experiments. Evaluation of effects have been based on results observed two weeks after tumor inoculation, except for sarcoma 180 solid form. Because of the rapid rate of growth of this tumor, observations were made after one week.

It is clear that the repeated injections of 1000 mg./Kg./day of actinobolin had a complete destructive effect on sarcoma 180 ascites tumor, and Ehrlich ascites carcinoma, but it had only slight inhibitory effect on the respective solid tumors. Actinobolin had a marked inhibitory effect on carcinoma 1025, glioma 26 (brain tumor), and Walker carcinosarcoma 256. It had a moderate inhibitory effect on adenocarcinoma E 0771; a slight inhibitory effect on Miyono adenocarcinoma, Friend virus leukemia, Jensen sarcoma, and Murphy-Sturm lymphosarcoma. There was no inhibition of sarcoma T 241, Lewis bladder carcinoma, Ridgway osteogenic sarcoma, Mecca lymphosarcoma, Gardner lymphosarcoma, and Harding-Passey melanoma.

## DISCUSSION

It is interesting to note that actinobolin was particularly active against the ascites tumors. Among 20 crystalline or purified antibiotics tested previously,<sup>15</sup> the follow-

ing five antibiotics also possessed destructive actions on the ascites tumors. These are actinomycins (C, D, and J), fumagillin, sarkomycin, carzinophillin, and mitomycin C.

Because actinobolin had a definite inhibitory effect on 4 out of 15 solid tumors, a comparison of its antitumor activity with that of 3 other antibiotics which we have studied recently<sup>13, 15</sup> might be of interest. These are actinomycin D, kanamycin, and mitomycin C, all isolated from strains of *Streptomyces*. From the tabulated data in table II, it is evident that antitumor activity of actinobolin and actinomycin D are essentially the same with the exception that actinobolin had a marked inhibitory effect on glioma 26 and Walker carcinosarcoma 256; actinomycin D had no inhibitory effect on these tumors. On the other hand, actinomycin D had a destructive action on Ridgway osteogenic sarcoma, whereas actinobolin was inactive.

A new antifungal antibiotic, kanamycin, demonstrated no inhibitory effect on any of the 22 tumors, including two ascites tumors. Mitomycin C, isolated from *Streptomyces caespitosus*<sup>2</sup> showed a marked to complete inhibitory effect on 15 of 24 various tumors. Such marked differences in effectiveness against various tumors again suggest the potential utility of the tumor spectrum as a means for evaluating new chemotherapeutic agents.<sup>16</sup>

Although clinical trials with a limited number of materials of microbial origin have not been so successful as had been anticipated on the basis of animal experimentation, these studies do give encouragement for the aggressive pursuit of anti-tumor antibiotics.

#### SUMMARY

Actinobolin had a marked inhibitory or destructive effect on carcinoma 1025, glioma 26, Walker carcinosarcoma 256, sarcoma 180 ascites tumor, and Ehrlich ascites carcinoma, and a moderate inhibitory effect on adenocarcinoma E 0771.

The comparison of the antitumor effects of actinobolin, actinomycin D, kanamycin, and mitomycin C is given.

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#### BIBLIOGRAPHY

1. DI PAOLO, J. A.; MOORE, G. E., AND NIEDBALA, T. F.: The influence of actinomycin D on animal and human tumors, *Proc. Am. A. Cancer Research* 2:195, 1957.
2. HATA, T.; SANO, Y., SUGAWARA, R.; MATSUMAE, A., KANAMORI, K.; SHIMA, T., AND HOSHI, T.: Mitomycin, a new antibiotic from *Streptomyces*, *J. Antib.* 9:141-146, 1956.
3. MANEY, B. E.: The hematological effects of TEM, Thio-TEPA, CB 1348 and actinomycin D in cancer patients, *Proc. Am. A. Cancer Research* 2:324, 1958.
4. MOORE, G. E.; KONDO, T., AND BADILLO, J.: Clinical trial of actinomycin D, *Proc. Am. A. Cancer Research* 2:329, 1958.
5. OTTO, H.: Erste erfahrungen mit sanamycin (Aktinomycin C) an lymphogranulomatosen und Leukamien, *Ztschr. f. ges. inn. Med.* 10:913-918, 1955.
6. PINKEL, D.: Actinomycin D in childhood cancer, *Proc. Am. A. Cancer Research* 2:335, 1958.
7. REILLY, H. C.; STOCK, C. C.; BUCKLEY, S. M., AND CLARKE, D. A.: The effect of antibiotics upon the growth of sarcoma 180 in vivo, *Cancer Research* 13:684-687, 1953.
8. SCHMIDT, H.; LOOSEN, H., AND HEINEN, W.: Actinomycin C (Sanamycin) in treatment of malignant tumors and lymphogranulomatosis, *Deutsche med. Wchnschr.* 80:140-143, 1955.
9. SHIMADA, N.; ISHII, R.; SATO, Y.; HATORI, T.; FUKUI, M.; KUBOTA, K.; SUKIE, K.; NOGUCHI, T., AND TAKEISHI, T.: Experimental and clinical studies on mitomycin A and X, *Chemotherapy* 4:305-306, 1956.

10. SHIRAHARA, Y.: Present status of antitumor chemotherapy in Japan, *Bull. de la Societe Internat. de Chirurgie*. In press.
11. SUGIURA, K.: Effect of various compounds on the Ehrlich ascites carcinoma, *Cancer Research* 13:431-441, 1953.
12. SUGIURA, K., AND STOCK, C. C.: Studies in a tumor spectrum. I. Comparison of the action of methyl bis(2-chloroethyl)amine and 3-bis(2-chloroethyl)aminomethyl-4-methoxymethyl-5-hydroxy-6-methylpyridine on the growth of a variety of mouse and rat tumors, *Cancer* 5:382-402, 1952.
13. SUGIURA, K., AND STOCK, C. C.: The effect of mitomycin C on a spectrum of tumors, *Proc. Am. A. Cancer Research* 2:350, 1958.
14. SUGIURA, K., AND STOCK, C. C.: Effects of compounds on the Friend mouse virus leukemia, *Proc. 7th Internat. Cancer Congress*. London, July 6-12, 1958, p. 187.
15. SUGIURA, K.; STOCK, C. C.; REILLY, H. C., AND SCHMID, M. M.: Studies in a tumor spectrum. VII. The effect of antibiotics on the growth of a variety of mouse, rat, and hamster tumors, *Cancer Research* 18:66-77, 1958.
16. SUGIURA, K., AND SUGIURA, M. M.: Tests of compounds against various mouse and rat tumors, *Cancer Research* 18:246-318, 1958.
17. SUKIE, K.; TAKEISHI, T., AND NOGUCHI, T.: Clinical trials of mitomycin C (antitumor substance), *Chemotherapy* 5:223-224, 1957.
18. TAN, C. T. C.; MURPHY, M. L.; DARGEON, H. W., AND BURCHENAL, J. H.: Clinical effects of actinomycin D, *Proc. Am. A. Cancer Research* 2:254-255, 1957.
19. TAPIE, J.: Resultats de l'emploi de l'actinomycine C en therapeutique (Results of the therapeutical use of actinomycin C), *Presse méd.* 63:1684, 1955.

# The Effects of Actinobolin on Transplanted Mouse Leukemias

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A new antibiotic with activity against a broad spectrum of microorganisms, for which the generic name actinobolin has been proposed was reported by Pittillo et al<sup>1</sup> in filtrates of a *Streptomyces*. The crude microbiological filtrate containing this compound was first tested by Reilly et al<sup>2</sup> against sarcoma 180 and was found to be inactive in the largest quantity (0.5 ml. twice daily intraperitoneally) that was employed. Because of the antimicrobial activity, Haskell et al<sup>3</sup> isolated the pure compound from the filtrate. Further work on the characterization of the antibiotic is in progress at the Parke, Davis Laboratories and will be reported. This purified preparation was retested against sarcoma 180 by Reilly et al<sup>2,4</sup> and found to be active (table I). For this reason, it has been studied against a broad spectrum of transplanted mouse leukemias, the results of which are herewith reported.

## METHOD

The technique for evaluation of the chemotherapeutic activity of a given drug by means of its ability to prolong the survival time of mice with transplanted leukemia has been described previously.<sup>5</sup> In a typical experiment approximately one hundred mice were injected intraperitoneally with 0.1 ml. of saline suspension of leukemic cells so diluted that 0.1 ml. contained one million cells. Twenty-four hours after inoculation, the mice were divided into comparable groups of 10 mice each with one set of controls and the remaining nine groups treated intraperitoneally daily or three times weekly for a 20 day period with the compounds under study. The mice were observed for the development of leukemia and autopsied at death. If gross evidence of leukemia was not conclusive, microscopic sections were taken. The mean survival times of treated and control mice were compared. The rationale behind the various steps of this technique has been discussed in detail in prior publications.<sup>5,6</sup>

Many of these studies were done on the sixty-sixth to eighty-fifth transplant generations of leukemia B82, which originated as a spontaneous leukemia in a C58 mouse in October, 1953. This transplanted leukemia kills in 10 to 15 days with an elevation of the white blood count to the 50,000 to 100,000 cells/cu. mm. level and tremendous enlargement of liver and spleen, and some enlargement of lymph nodes. This leukemia was also injected subcutaneously (B82T) to give local tumors. In this case, the mice were sacrificed at 14 days and the tumors weighed. Leukemias B82 and B8174 were carried in F<sub>1</sub> hybrids of the Bagg albino female × C58 male cross. Leukemia L1210,<sup>7</sup> originally supplied by Lloyd Law, and L1210/A, L1210/MP, L1210/AG, L1210/AMPD,<sup>8</sup> and a line of L1210 made resistant to actinobolin were also studied in DBA mice or F<sub>1</sub> hybrids thereof. In addition to these, actinobolin was tested in the same mice against the following

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TABLE I  
Inhibition by Actinobolin Sulfate of Sarcoma 180 in Mice<sup>2</sup>

Dose Gm./Kg./day	Effect*	$\Delta$ wt. Gm. 7 days	Deaths
2.0	$\pm^+$	—4.5	2
		—1.0	10
1.0	$\pm^-$	—2.0	0
		—1.0	15
0.5	—	—1.0	0
		—0.5	10

\*  $\pm^+$  = Average diameter tumors in treated mice  $\frac{1}{4}$  to  $\frac{1}{2}$  that of control;  $\pm^-$  = average diameter tumors  $\frac{1}{2}$  to  $\frac{3}{4}$  controls; — = average diameter of tumors  $> \frac{3}{4}$  that of controls.

leukemias kindly supplied by Michael Potter: chloroleukemia P1081; mast-cell leukemia P815; reticulum-cell leukemia P329; and acute lymphocytic leukemia P388.<sup>9, 10</sup>

## RESULTS

Figure 1 is a scatter diagram demonstrating the effect of actinobolin in prolonging the survival time of mice with L1210 leukemia. At the usual dose of 800 mg./Kg. daily the mean survival time was increased from 11.3 days in the controls to 20.3 days in the treated mice. As can be seen in table II, actinobolin at a dose of 800 mg./Kg. daily caused a significant prolongation of survival time or inhibition of tumor growth in leukemias L1210, P1081, P388, B82, B82T, B8174T but had somewhat less effect on P329 and P815. Figure 2 demonstrates that it is equally effective against the strains of L1210 made resistant to amethopterin (L1210/A), to mercaptopurine (L1210/MP), or to both amethopterin and mercaptopurine (L1210/AMPD).<sup>8</sup> As can be seen, either single or double resistance to mercaptopurine and amethopterin had no effect on the actinobolin activity. To elucidate further the mechanism of action of actinobolin, a line of L1210 leukemia originally very sensitive to this compound was passed through animals treated with actinobolin, 800 mg./Kg. daily. A certain degree of resistance had been developed by the eleventh generation. By the thirty-fifth generation, the resistance was apparently complete. At this time, the line was studied for its response for various of the conventional antileukemic agents, and as can be seen in figure 3, although it was completely resistant to actinobolin, there was no cross resistance to amethopterin, diazo-oxo-norleucine, or 5-fluorouracil. Actinobolin was also studied for its effect

FIG. 1. The effect of actinobolin on the survival time of mice with leukemia L1210 is shown.

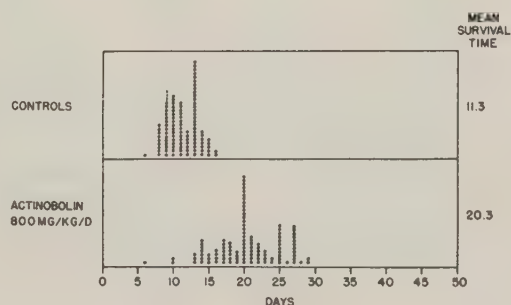


TABLE II  
Effect of Actinobolin on a Spectrum of Transplanted Leukemias

Leukemia	Controls		Actinobolin 800 mg./Kg./d		% increase	Δ weight	% inhibition
	Mean	SD	Mean	SD			
Survival time							
B 82 ip	14.7	± 3.2	30.8	± 3.6	+109	—0.8	
L1210	9.9	± 1.7	22.1	± 5.9	+123	—2.	
P1081	22.6	± 2.8	58	± 37.3	+155	+0.5	
P815	16.8	± .48	24.2	± 1.4	+ 44	+0.7	
P388	13.1	± 2.7	26.6	± 1.1	+103	—0.5	
P329	15.2	± 1.3	21.6	± 1.6	+ 42	+2.5	
Tumor weight							
B 82T sc	1518	± 110	253	± 193		—2.3	+83
B 8174T sc	811	± 214	41	± 38.1		+1.	+95

on *Streptococcus faecalis* ATCC 8043 growing in pour plates of Difco folic acid assay agar with 5 milligram/ml. of added pterolylglutamic acid. When 0.08 ml. of a 10 mg./ml. solution of actinobolin was placed on a 12.7 mm. diameter filter paper disc there was a zone of inhibition of approximately 30 mm. in diameter. Because actinobolin had been reported to be a strong chelating agent for iron and aluminum,<sup>3</sup> the effects of metal ions on this inhibition of *Str. faecalis* were studied. As can be seen in figures 4 and 5, ferric chloride at 25 mg./ml., and aluminum chloride at 16.0 mg./ml. interfered with this inhibitory effect. In other studies magnesium sulfate at saturated solution and ammonium molybdate at 133 mg./ml. also were active but ferrous sulfate, calcium gluconate, cadmium chloride, copper acetate, cobalt chloride, zinc acetate, and barium chloride did not interfere with this inhibitory effect. When these metal salts in maximum tolerated doses and standard doses of actinobolin were given intraperitoneally, there was no inhibition of the antileukemic effects of the latter. In fact, there was some indication that the combination of ferric chloride and actinobolin was more toxic than either compound alone.

## DISCUSSION

Actinobolin is a compound which displays a high degree of activity against a wide spectrum of transplanted mouse leukemias. It is as effective against lines made resistant to amethopterin, to mercaptopurine, or to a combination of the two as against the parent lines. Similarly, in a line made resistant to actinobolin, there was no cross resistance to either amethopterin, mercaptopurine, the fluorinated pyrimidines, or actinomycin. The fact that it has been shown to be a strong chelating agent for iron and aluminum suggested that this might be the mode of action, but

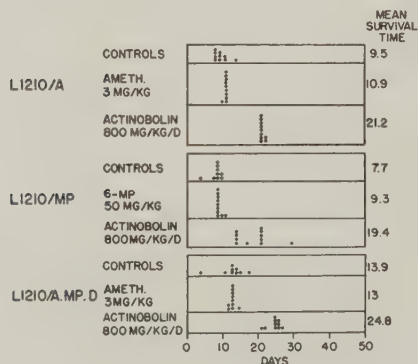


FIG. 2. The effect of actinobolin on resistant leukemias is illustrated. All doses were given three times a week unless otherwise specified. L1210/A, resistant to amethopterin; L1210/MP, resistant to mercaptopurine; L1210/AMPD, resistant to both amethopterin and mercaptopurine.

FIG. 3. The effect of various antileukemic agents on the thirty-fifth generation of a line of L1210 leukemia resistant to actinobolin is shown. DON = diazo-oxo-norleucine. All doses were given three times a week unless otherwise specified.

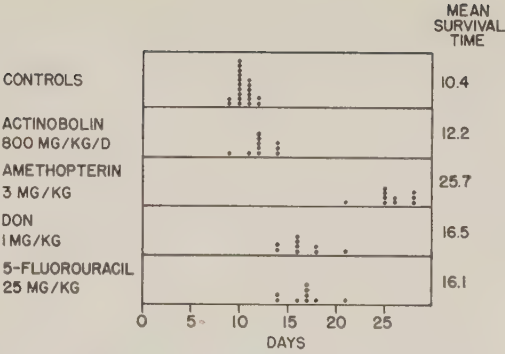


FIG. 4. The effect of ferric chloride and aluminum chloride on the inhibitory effect of actinobolin on *Streptococcus faecalis* is shown.

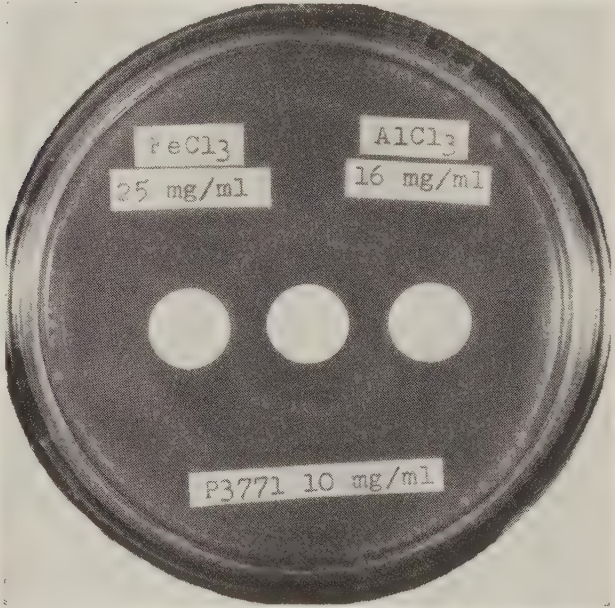
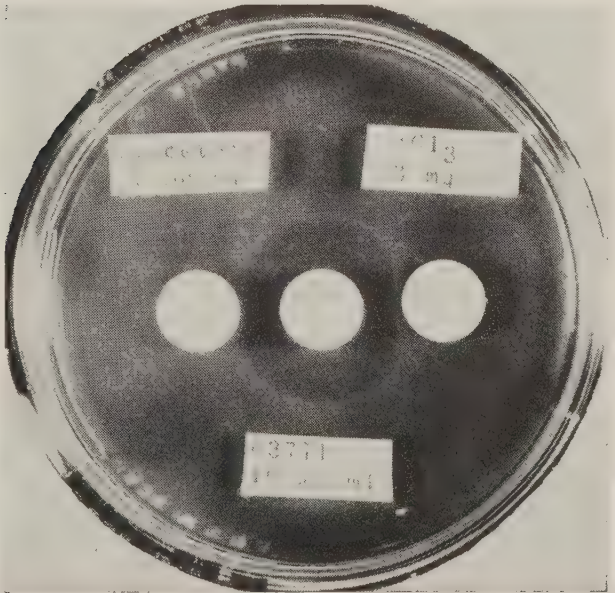


FIG. 5. The effect of copper acetate and aluminum chloride on the inhibitory effect of actinobolin on *Streptococcus faecalis* is illustrated.



when the drug was given as the preformed aluminum chelate or in combination with rather large doses of iron or aluminum salts the fact that it maintained its activity would suggest that it is not working through the mechanism of chelation. Further, the fact that another chelating agent, methylglyoxal bis(guanylhydrazone), which had been shown previously to be active against L1210 leukemia by Freedlander and French,<sup>11</sup> is still active against the L1210 leukemia made resistant to actinobolin and also that actinobolin is active against a line of L1210 leukemia<sup>11</sup> made resistant to the glyoxal bis(guanylhydrazone) might also be suggestive evidence that this compound is not acting by virtue of being a chelating agent. When this drug is given in combination with half the effective dose of amethopterin, 6-mercaptopurine, thioguanine, diazo-oxo-norleucine, actinomycin D, thioguanosine, 5-fluoroorotic acid, 5-fluorouracil, urethane, and actinomycin F<sub>1</sub>, it has demonstrated no synergistic activity. From these cross resistance and reversal studies, it would appear that this compound is acting by mechanisms different from those of these previously studied chemotherapeutic agents.

#### SUMMARY

Actinobolin has been demonstrated to possess antileukemic activity against a wide spectrum of transplanted mouse leukemias. Strains of leukemia L1210 that have developed resistance to amethopterin or mercaptopurine or both are not resistant to actinobolin nor is a strain that has developed resistance to glyoxal bis(guanylhydrazone). A strain of L1210 that has developed a high degree of resistance to actinobolin is still sensitive to amethopterin, mercaptopurine, diazo-oxo-norleucine, the fluorinated pyrimidines, and methylglyoxal bis(guanylhydrazone). These studies on the cross resistance would suggest that this new antibiotic is acting by a mechanism differing from that of the previously known antileukemic agents. Clinical trials of this compound are now in progress.

#### BIBLIOGRAPHY

1. PITILLO, R. F.; FISHER, M. W.; McALPINE, R. J.; THOMPSON, P. E., AND EHRLICH, J.: Actinobolin, a new broad-spectrum antibiotic. Origin and biologic evaluation. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 497.
2. REILLY, H. C., AND CLARKE, D. A.: Effect of actinobolin against sarcoma 180. Personal communication, Oct., 1956.
3. HASKELL, T. H., AND BARTZ, Q. R.: Actinobolin, a new broad-spectrum antibiotic, isolation and characterization. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 505.
4. SUGIURA, K., AND REILLY, H. C.: The effect of actinobolin on a spectrum of tumors. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 522.
5. BURCHENAL, J. H.; JOHNSTON, S. F.; BURCHENAL, J. R.; KUSHIDA, M. N.; ROBINSON, E., AND STOCK, C. C.: Chemotherapy of leukemia IV. Effect of folic acid derivatives on transplanted mouse leukemia. *Proc. Soc. Exper. Biol. & Med.* 71:381-387, 1949.
6. BURCHENAL, J. H.; LESTER, R. A.; RILEY, J. B., AND RHOADS, C. P.: Studies on chemotherapy of leukemia I. Effect of certain nitrogen mustards and carbamates on transmitted mouse leukemia, *Cancer* 1:399-412, 1948.
7. LAW, L. W.; DUNN, T. B.; BOYLE, P. J., AND MILLER, J. H.: Observations on the effect of a folic-acid antagonist on transplantable lymphoid leukemias in mice, *J. Nat. Cancer Inst.* 10:179-192, 1949.
8. BURCHENAL, J. H., AND HOLMBERG, E. A. D.: The utility of resistant leukemias in screening for chemotherapeutic activity, *Ann. New York Acad. Sc.* Oct. 1958. In press.
9. DUNN, T., AND POTTER, M.: A transplantable mast-cell neoplasm in the mouse, *J. Nat. Cancer Inst.* 18:587-601, 1957.
10. POTTER, M.: Personal communication, 1958.
11. FREEDLANDER, B. L., AND FRENCH, F. A.: Carcinostatic action of polycarbonyl compounds and their derivatives. II. Glyoxal bis(guanylhydrazone) and derivatives, *Cancer Research* 18:360-364, Apr., 1958.

# Clinical Trials of Mitomycin C, a New Antitumor Antibiotic

## Preliminary Report of Results Obtained in 82 Consecutive Cases in the Field of General Surgery

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Hata et al<sup>1</sup> reported first that mitomycin, a new antitumor antibiotic isolated from culture broth of *Streptomyces caespitosus*,<sup>13</sup> is active against several kinds of gram-positive and gram-negative bacteria, a few kinds of viruses and *Ascaris*<sup>2</sup> and it possesses also extraordinarily strong tumor-inhibiting effects on Yoshida ascites sarcoma and Ehrlich carcinoma, both in ascitic and solid forms. They isolated two fractions of mitomycins, A and B. However, Wakaki et al<sup>26</sup> could not identify the same fractions in their process of culturing the mitomycin-producing strain, but they isolated a new active substance in crystalline form, tentatively called mitomycin X and later renamed mitomycin C by suggestion of Hata. Marumo et al<sup>7</sup> and Wakaki et al<sup>27</sup> reported recently other kinds of mitomycins and described their physical properties.

The tumor-inhibiting activity of mitomycin C has been clearly confirmed by a series of experimental studies, both in Japan<sup>3, 7, 8, 11-13, 18, 22, 23, 25</sup> and in the United States.<sup>6, 16, 17</sup> Pharmacological study of this new antibiotic and its action mode is now being undertaken by some investigators in Japan.<sup>19-21</sup>

Shimada et al<sup>13</sup> and Sukie et al<sup>18</sup> tried mitomycin clinically first and reported some beneficial effects on 13 cases of advanced cancer. Since one of us (Y. S.) reported therapeutic experiences with this drug at the International Congress of Surgery in Mexico in 1957, we have treated 82 consecutive cases of patients suffering from various kinds of cancer with mitomycin C. The present report is mainly based upon this group of patients who were treated from August 1, 1957, to June 30, 1958.<sup>24</sup>

### DOSAGE AND PATIENTS

*Drug and Dosage.* One mg. of mitomycin C crystal in a vial was dissolved by shaking in 5 ml. of distilled water at 60 C. to be used for various injections. The antibiotic was administered intravenously in 64 cases, 4 of whom were treated by combined use with direct infiltrative injection of the drug into the tumor tissue. The remaining 18 cases were administered intra-arterially through a polyethylene tube indwelt in the supply stem artery to the malignant lesion. The drug was administered daily in a dose of 1 to 5 mg. (average 2 to 3 mg.). For intravenous injection it was used in a total dose of 2 to 95 mg. (average 35 mg.) in a period of 3 to 82 days (average 24 days); for intra-arterial use it was administered in a total dose of 3 to 156 mg. (average 49 mg.) in a period of 2 to 82 days (average 39 days). And for local infiltration into the tumor tissue it was administered daily in a dose of 1 to 2 mg. in a total dose of 21 to 84 mg. (average 54 mg.) in a period of 29 to 56 days (average 43 days). The antibiotic was administered as much as possible in total dose until the leukocyte count of the patient was reduced to 2000 to 2500 and then was discontinued in expectation of its recovery.<sup>24</sup>

*Patients.* The patients treated with mitomycin C were almost all adults (15 to 73 years of age), the average being 53 years. There were 46 men and 36 women.

Their clinical diagnosis is classified as: stomach cancer, 46; rectal cancer, 9; breast cancer, 7; cancer of jaw, 5; sarcoma of maxilla, 3; cancer of tongue, 2; and one each of cancer of the liver, thyroid, larynx, lung, uterus, and intestine; and one each of seminoma, branchiogenous cancer, reticulosarcoma, and Hodgkin's disease. Ten patients (12 per cent) were given mitomycin C to prevent recurrence of cancer following either radical or suspected incomplete excisional operations; but in 72 cases (88 per cent), the cancers were so advanced or generalized that either radical surgery was impossible or only palliative surgery was performed, followed by antitumor chemotherapy. Histological classification of the lesions listed in table I shows a predominance of adenocarcinoma (66 per cent).

#### ILLUSTRATIVE CASE HISTORIES

*Case 1.* K. N., a 51 year old Japanese man had cancer of the jaw. The patient had slight difficulty in chewing in August, 1956, and was placed on irradiation therapy under a diagnosis of cancer of the right jaw. There was considerable regression of symptoms, but they recurred in May, 1957, when he was first seen at our Clinic. Biopsy taken out of an indurated ulcer in an area of 3 x 4 sq. cm. at the site of the right under wisdom tooth revealed basal-cell cancer. This showed no definite reaction to intra-arterial triethylenethiophosphoramide administered with a daily dose of 10 to 15 mg., in a total of 220 mg. However, intravenous injection of mitomycin C in a daily dose of 1 mg. resulted in gradual softening of the tumor; there was reduction of its size up to complete disappearance and improvement of chewing difficulty, after a total use of 69 mg. of mitomycin C over a period of 81 days. On December 10, 1957, when the patient was discharged from the hospital, at the site of the previously ulcerated tumor, there was scar tissue that histologically showed only fibrous tissue, without any sign of cancer cells. A recent check-up of the patient revealed neither sign of recurrence nor metastasis, and he has been on full duty in complete health for nine months since discontinuance of the drug.

*Case 2.* Y. K., a Japanese man of 60 years of age had cancerous peritonitis originating from stomach cancer. The patient developed general fatigue and weight loss about the end of 1957 and was referred first to our Clinic on May 6, 1958, when he was severely starved, showing a frog belly with a tumor in a size of man's fist in the epigastrium. He was started on intravenous mitomycin C therapy with a daily dose of 2 mg. and showed immediate improvement in general feeling, gain in appetite, increase in urinary secretion, and over a period of 28 days showed a reduction of ascites fluid and a decrease of his tumor to thumb size. After completion of a total dose of 56 mg. of mitomycin C, the patient was discharged from the hospital to the care of a family physician who was to observe his postchemotherapeutic course. A fluoroscopy prior to mitomycin C therapy revealed a remarkable stenosis of the pylorus, and there was a gradual response to 26 mg. of mitomycin C in total, resulting in normal passage (figs. 1, 2).

*Case 3.* Y. M. was a Japanese man of 56 years of age. The patient was placed on medical treatment in the summer of 1954 under a diagnosis of gastric ulcer and was referred first to

TABLE I  
*Histological Classification of the Lesions Treated with Mitomycin C*

Classification	Number of cases	Percentage
Adenocarcinoma	39	66
Undifferentiated carcinoma	9	15
Squamous-cell carcinoma	4	7
Mucoid carcinoma	3	5
Basal-cell cancer	1	2
Reticulosarcoma	2	3
Round-cell sarcoma	1	2

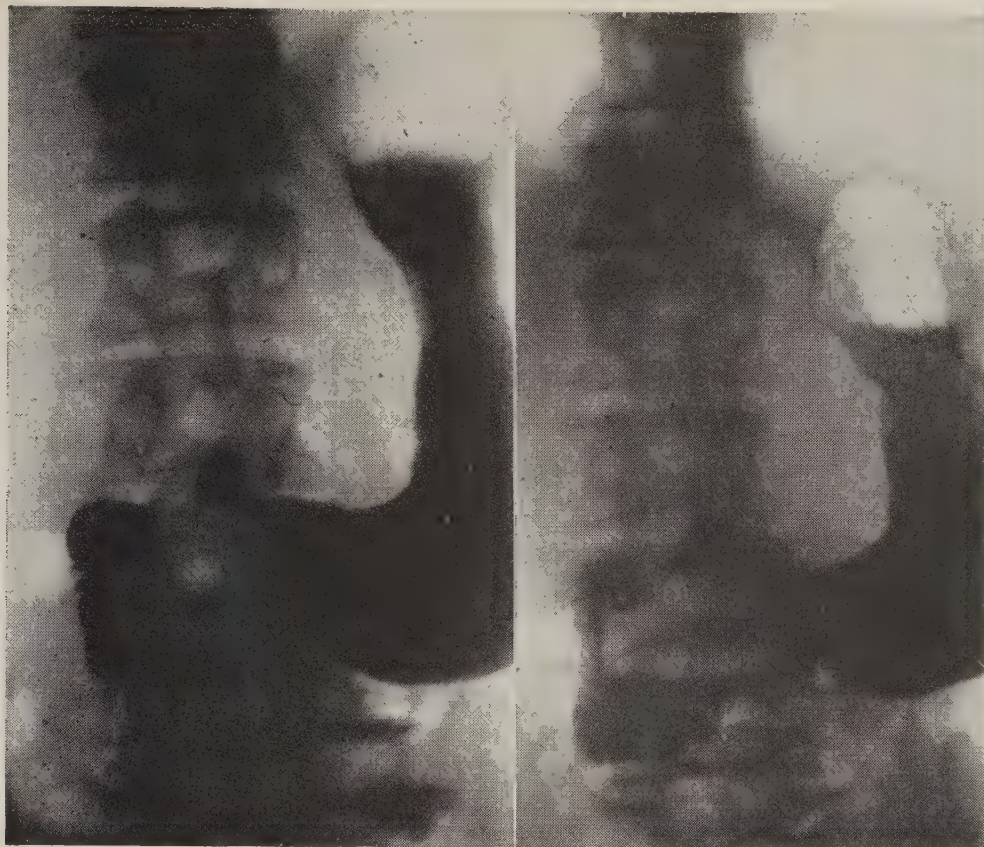


FIG. 1. (Left) Case 2, Y. K. A fluoroscopy prior to mitomycin C therapy showed a remarkable stenosis of the pylorus.

FIG. 2. (Right) There was a gradual response to 26 mg. of intravenous mitomycin C, resulting in normal passage.

our Clinic on September 19, 1957. He underwent a laparotomy which revealed an inoperable tumor (adenocarcinoma) at the pylorus complicated by intraperitoneal diffuse dissemination of the cancer, which permitted only gastrojejunostomy. A polyethylene tube was inserted into the abdominal aorta through the right femoral artery, fixing its intra-aortic stump at the branching site of celiac axis for the purpose of following administration of antitumor chemotherapeutic agent. Intra-arterial administration of mitomycin C was started on September 26, 1957, and a dose of 1 mg. in 24 hours was continued up to total dosage of 36 mg. during 48 days without untoward side effects. The patient gained 6 Kg. of body weight during the chemotherapy and was discharged on November 16, 1957. When he was checked up in July, 1958, he was completely well, without any sign of gastric cancer and had been on full duty since his discharge from the hospital.

We would like to present another pertinent case which showed clear histological responses to mitomycin C therapy.

*Case 4.* K. W., a Japanese man of 58 years of age had recurrent gastric cancer. He underwent first in October, 1956, a subtotal gastrectomy in our Department of Surgery and was reoperated upon on September 9, 1957, when the recurrent tumor at the site of gastrojejunostomy was palliatively resected, combined with ileosigmoidostomy. He was started on intra-arterial mitomycin C therapy with a daily dose of 2 mg. through a polyethylene tube indwelt in the abdominal aorta from the right femoral artery. Figures 3 and 4 illustrate the biopsies taken from the lesion prior to the antibiotic therapy and after completion of a total administration of 100 mg. of the drug, respectively. These microphotographs clearly show that the gastric adenocarcinoma has been thrown into severe degenerative changes associated with vigorous proliferation of connective tissue.

Clinical application of mitomycin C resulted in immediate improvement in the general condition of almost all the patients, particularly in the intra-arterially administered group, as evidenced by the comfortable general feeling and gain in both appetite and body weight. In table II are listed changes in clinical symptoms and signs in 82 cases following chemotherapy with either intravenous or intra-arterial mitomycin C. Among these, the most remarkable is regression of tumors (30 per cent), including a complete disappearance confirmed both clinically and histologically in case 1. Reduction of ascites combined with increased urinary secretion is also one of the beneficial clinical responses. For example, one of the patients with cancerous peritonitis showed a reduction of his abdominal circumference in a length of 13 cm. with increased daily urine volume from 400 to 1800 ml. within 10 days following intravenous mitomycin C therapy.

A patient with advanced bronchogenic cancer experienced a decrease in cough and chest pain within a week following commencement of intravenous chemotherapy. Alleviation up to complete relief of pain was also noted in 6 other cases, but intractable pain of the patients with extremely advanced cancer was not relieved by mitomycin C therapy.

Ten cases were given mitomycin C (1 to 2 mg. daily, 9 to 43 mg. in a total dose) to prevent recurrence after radical operations, and none showed signs of cancer when they were checked up 8 to 10 months after the discontinuance of the antibiotic.

Four cases who received mitomycin C directly into the tumor tissues, combined

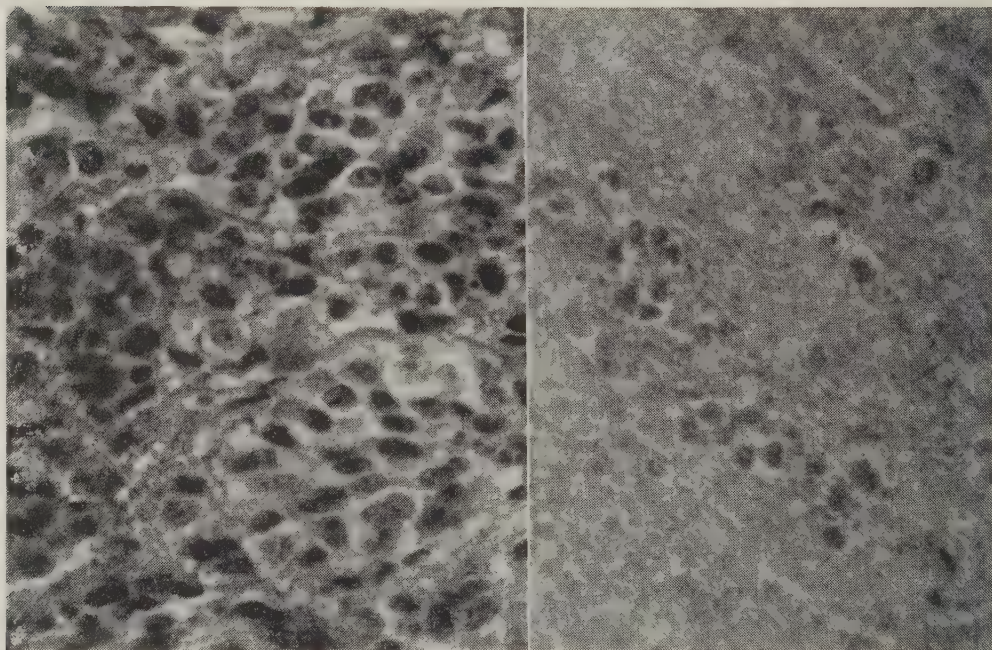


FIG. 3. (Left) Case 4, K. W. Biopsy taken prior to mitomycin C therapy shows gastric adenocarcinoma.

FIG. 4. (Right) Biopsy taken after a total administration of 100 mg. of intra-arterial mitomycin C illustrates that the gastric adenocarcinoma has been thrown into severe degenerative changes associated with vigorous proliferation of connective tissue.

TABLE II  
*Clinical Effects of Mitomycin C Therapy*

Classification of clinical signs and symptoms	No. of cases		Total
	Intravenously administered	Intra-arterially administered	
Regression of tumor, including complete disappearance	18	7	25
Gain of body weight	4	3	7
Reduction of ascites, increase of urinary secretion	4	2	6
Alleviation, up to complete relief of pain	5	1	6
Alleviation of nausea and vomiting	4	2	6
Improvement of chewing difficulty	4		4
Softening and necrosis of tumor	3		3
Mitigation of cough and sputum	1		1

with intravenous administration, responded by a softening and necrosis of the tumors: but all of them died after total use of 29 to 56 mg. of mitomycin C.

#### SIDE EFFECTS

*Leukopenia.* Among untoward side effects following mitomycin C therapy, leukopenia was experienced in the majority of patients; that is, 84 per cent in the intravenous group and 67 per cent in the intra-arterial group. The leukocyte count of the peripheral blood decreased an average of 45 per cent in the intravenous group and 31 per cent in the intra-arterial group after a three week period of medication, thereby resulting in a steeper decrease in the former. Table III shows average course of leukopenia co-related with administering routes, daily dose and period of medication. However, the leukopenia provoked by mitomycin C therapy was recovered within three weeks after the discontinuance of the antibiotic (table IV). Moreover, it was comparatively faster in the intra-arterial than in the intravenous group. Leukocyte analysis revealed that the neutrophils were reduced at first, indicating a relative rise of lymphocytes.

*Other Side Effects.* In general, untoward side effects other than leukopenia were relatively rare and not so severe as indicated by anorexia (8), fever (3), general fatigue (1), and subcutaneous bleeding (1); but it has not been established whether some of them were directly related to medication with mitomycin C or to primary cancer lesions.

Since our first patient showed slight bleeding tendency following the use of mitomycin C, we examined very carefully the platelet count of the subsequent patients (14) and estimated their bleeding and clotting times, but the results were non-contributory. Erythrocyte count and hemoglobin assay revealed no changes in

TABLE III  
*Development of Leukopenia Following Mitomycin C Therapy\**

Period week	Intravenous					Intra-arterial				
	Daily dose, mg.					Daily dose, mg.				
	1	2	3	5	Average	1	2	3	5	Average
1	22	22	23	30	24	17	20	20	24	20
2	32	40	41	55	42	21	24	26	30	25
3	40	42	54		45	28	32	34	39	31

\* The figures indicate reduction rate of leukocyte count in percentage.

TABLE IV  
*Recovery of Leukopenia Provoked by Mitomycin C\**

Lapse of time after discontinuance of mitomycin C, wk.	Administering routes	
	Intravenous	Intra-arterial
1	29	36
2	53	80
3	80	100

\* The figures indicate recovery rate of leukopenia in percentage.

42 per cent of the patients and increased or decreased values, each in 29 per cent. Functional tests of liver and kidney and urine assay revealed no changes provoked by the antibiotic.

#### DISCUSSION

Antitumor activity of mitomycin C has been confirmed experimentally by some investigators, both in Japan and in the United States, among whom Sugiura has recently emphasized its extraordinarily strong tumor-inhibiting activity on animal cancers. We have tried this new antitumor antibiotic clinically on 82 consecutive cases of cancer, confirming its anticancerous activity and low toxicity on human beings. Unfortunately, we have had yet no experience to treat cases of leukemia and chorioepithelioma with this antibiotic in the field of general surgery. We hope to treat such cases in the future.

Among side effects of mitomycin C, leukopenia is a predominant one. However, it is relatively easily recovered within three weeks, especially after completion of intra-arterial administration. Intra-arterial application through a polyethylene tube indwelt in the supply stem artery to the lesion is a method of choice as an administering route of antitumor chemotherapeutic agent. It makes it possible to distribute the drug to the lesion at a higher concentration and in a larger dose than other routes and facilitates therapeutic effects. In addition, it has been confirmed that some side effects, particularly leukopenia caused by the medicated drug are reduced by this method of administration, and recovery is accelerated.<sup>10</sup>

Some of the investigators<sup>4</sup> recognize shrinkage of spleen in animals who receive mitomycin. However, Sugiura et al<sup>16,17</sup> could not make the same observation, and neither could it be demonstrated at our institution.<sup>23</sup>

One of our patients treated with mitomycin C developed slight bleeding tendency that was not confirmed by coincidental findings of laboratory examinations. However, the problem of bleeding tendency caused by mitomycin C should be investigated further by both experimental study in animals and clinical use in human beings in the future. Some of tumor-inhibiting antibiotics, for example, sarkomycin,<sup>9</sup> has been proved to have a particular activity to change permeability of the wall of blood capillary in the tumor tissue, sometimes resulting in extensive hemorrhage and necrosis.

#### CONCLUSION

Tumor-inhibiting activity of mitomycin C has been confirmed clearly by a series of experimental studies on animal cancer and by clinical trials on 82 cases with

cancer experienced in the field of general surgery, its toxicity having been proved to be very low. This antitumor antibiotic seems to be so promising and beneficial that it should be tried in wider fields of antitumor chemotherapy to evaluate its therapeutic effect. The authors would like to emphasize also the advantage of intra-arterial use of the antitumor chemotherapeutic agent through a polyethylene tube indwelt in the supply stem artery as a method of choice to facilitate therapeutic effects and to prevent toxic side effects caused by the drug.

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#### BIBLIOGRAPHY

1. HATA, T.; SANO, R.; SUGAWARA, R.; MATSUMAE, A.; KANAMORI, K.; SHIMA, T., AND HOSHI, T.: Mitomycin, a new antibiotic from *Streptomyces*, J. Antib., Ser. A. 9:141-146, 1956.
2. HATA, T.; MATSUMAE, A.; OWADA, K., AND SANO, Y.: Study on treatment with mitomycin against experimental infections, Chemotherapy 4:295-296, 1956.
3. ITO, I.; TAGUCHI, T.; AKAGI, A.; YAMASHITA, E., AND YAMADA, M.: Study on antitumor activity of mitomycin X. I. Influence upon Ehrlich ascitic carcinoma. Personal communication, 1958.
4. KANAMORI, H.; SHIMA, T.; MORITA, C., AND HATA, T.: Studies on antitumor activity of mitomycin, J. of Antib., Ser. A. 10:120-127, 1957.
5. MARUMO, H.; WAKAKI, S.; TOMIOKA, K.; SHIMIZU, G.; KATO, E.; KAMADA, H.; KUDO, S., AND FUJIMOTO, Y.: On new fractions of mitomycins, an antitumor substance, Proceedings of Japanese Association of Agricultural Chemistry (Kyoto), 1958.
6. REILLY, H. C.; CAPPUCCINO, J. G., AND HARRISON, D. M.: Studies on mitomycin C, a tumor inhibiting antibiotic, Proc. Am. Cancer Research 2:338, 1958.
7. SAKAI, K., AND TERANAKA, T.: On antitumor activity of mitomycin against experimental animal tumors, Chemotherapy 5:222-223, 1957.
8. SAKAI, K.; YOSHIMURA, K.; HAYASHI, K.; MARUI, F.; KIMURA, T.; SAWADA, A., AND HASHIMA, K.: Studies on tumor inhibiting activity of mitomycin, Chemotherapy 5:322, 1957.
9. SAKAI, K.: Studies of antitumor effect of Sarkomycin, Arch. f. japan. Chir. 25:699-726, 1956.
10. SAKAI, K.: Studies on effect of intra-arterial administration of Nitrogen mustard N-oxide on rat tumor, Arch. f. japan. Chir. 25:727-734, 1956.
11. SHIBA, S.; ITO, I.; TAGUCHI, T., AND AKAGI, A.: Experimental study on mitomycins, Chemotherapy 5:322-323, 1957.
12. SHIMA, T.; KANAMORI, H.; HOSHI, T.; MORITA, C., AND HATA, T.: Study on treatment with mitomycin against experimental malignant tumors, Chemotherapy 4:304-305, 1956.
13. SHIMADA, N.; ISHII, R.; SATO, Y.; HATORI, T.; FUKUI, M.; KUBOTA, K.; SUKIE, K.; NOGUCHI, T., AND TAKEISHI, T.: Experimental and clinical studies on mitomycins A and X, Chemotherapy 4:305-306, 1956.
14. SHIRAHARA, Y.: Present status of antitumor chemotherapy in Japan, Bull. Soc. Internat. Chir. In press.
15. SUGAWARA, R., AND HATA, T.: Mitomycin, a new antibiotic from *Streptomyces*, II. Description of the strain, J. Antib., Ser. A. 9:147-151, 1956.
16. SUGIURA, K., AND STOCK, C. C.: The effect of mitomycin on a spectrum of tumors, Proc. Am. A. Cancer Research 2:350, 1958.
17. SUGIURA, K., AND STOCK, C. C.: Effect of compounds on the Friend mouse virus leukemia, Proc. 7th Internat. Cancer Congress (London) 187, 1958.
18. SUKIE, K.; TAKEISHI, T., AND NOGUCHI, T.: Clinical trials of mitomycin C (antitumor substance), Chemotherapy 5:223-224, 1957.
19. TAGUCHI, T., AND AKAGI, A.: Pharmacology of mitomycins. Personal communication, 1958.

20. TAGUCHI, T.; AKAGI, A., AND KONTANI, H.: Influence of mitomycin X upon biosynthesis of nucleoprotein of *E. Coli* B. Personal communication, 1958.
21. TAGUCHI, T., AND AKAGI, A.: Study on action mode of mitomycin X (I). Personal communication, 1958.
22. TERANAKA, T.: Evaluation of antitumor activity of mitomycin, *J. Antib., Ser. A.* 10:126, 1958.
23. TERANAKA, T.: Studies on antitumor activity of mitomycin X. I. Experimental study on tumor inhibiting activity of mitomycin X, *J. Osaka City Med. Center* 7:24-31, 1958.
24. TERANAKA, T.: Studies on antitumor activity of mitomycin C. II. Clinical trials of mitomycin C. Unpublished.
25. USUBUCHI, I.: Effect of mitomycins on experimental tumors. *Chemotherapy* 5:222, 1957.
26. WAKAKI, S.; MARUMO, H.; TOMIOKA, K.; SHIMIZU, G.; KATO, E.; KAMADA, H.; KUDO, S., AND FUJIMOTO, Y.: Isolation of new fractions of antitumor mitomycins. *Antib. & Chemo.* 8:228-240, 1958.
27. WAKAKI, S.; MARUMO, H.; TOMIOKA, K.; SHIMIZU, G.; KATO, E.; KAMADA, H.; KUDO, S., AND FUJIMOTO, Y.: On new fractions of mitomycins, an antitumor substance. *Proceedings of Japanese Association of Agricultural Chemistry (Kyoto)* 1958.

# Studies on the Carcinolytic Activity of Fumagillin and Some of Its Derivatives

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For the past three years this laboratory has been studying the anticarcinogenic activity of fumagillin and some of its derivatives. This report summarizes the experimental results obtained with these compounds and gives a preliminary report of clinical experience with fumagillin and fumagillin alcohol-I in a group of human cancers.

Fumagillin is a crystalline antibiotic isolated from a strain of *Aspergillus fumigatus*.<sup>1</sup> It is unique in being essentially inactive against bacteria and viruses but very effective for the treatment of intestinal amebiasis. Furthermore, Hanson and Eble<sup>2</sup> demonstrated that an antiphage, H-3, from a strain of *A. fumigatus* inhibited growth of *Staphylococcus aureus* 209 bacteriophage but had little antibacterial or antifungal activity. Fumagillin, whose structure is unknown, is a weak acid having an empirical formula of  $C_{57}H_{36}O_7$  with a molecular weight of 472 and a melting point around 192 C. The material is insoluble in water but soluble in organic solvents such as chloroform.

Hydrolysis with sodium hydroxide of the antibiotic fumagillin results in alcohol-I,  $C_{16}H_{26}O_4$ ,<sup>3,4</sup> and decatetraenedioic acid.<sup>5</sup> This alcohol moiety has been obtained in a crystalline form and has been shown to contain an epoxide group. Lithium aluminum hydride reduces alcohol-I to open the epoxide ring to form a crystalline diol, dihydroalcohol-Ib,  $C_{16}H_{28}O_4$ .<sup>3</sup> Further reduction of dihydroalcohol-Ib produces tetrahydroalcohol-Iab  $C_{16}H_{30}O_4$  and tetrahydroalcohol-Ib,  $C_{16}H_{30}O_4$ .<sup>3,6</sup> Reduction of alcohol-I, dihydroalcohol-Ib, and tetrahydroalcohol-Iab by lithium aluminum hydride in refluxing tetrahydrofuran produces more  $C_{16}$  triols.<sup>7</sup>

## MATERIALS AND METHODS

Fumagillin and the compounds derived from it were suspended in 0.5 per cent carboxymethylcellulose dissolved in water. For mouse tumor study suspensions were made so that a 20 Gm. mouse would receive 0.2 ml. injection of material twice daily. Swiss mice and dogs were used in some toxicity experiments.

Where possible, tests were carried out on microbial systems as well as animal tumors in vivo. The disc assay technique was used with *Bacillus subtilis*, *Escherichia*, *Aerobacter aerogenes*, and with *Clostridia* in Brewer anaerobic jars; ascites tumor cells maintained in agar pour plates<sup>8,9</sup> and induced respiratory mutants of *Saccharomyces carlbergensis* 4428, *S. cerevisiae* 1741, and *S. cerevisiae* var. *ellipsoideus*.<sup>9</sup>

In the cases involving tumor-bearing animals, intraperitoneal injections of 0.2 ml./20 Gm. mouse of material in carboxymethylcellulose were given twice daily beginning the day after tumor transplantation and continued for seven days in the case of the Ehrlich-<sup>2</sup> ascites, Krebs-<sup>2</sup> ascites, leukemia 1210, and sarcoma 180. In the cases in which solid carcinoma C3HBA and C-755 tumors were used, daily injections were continued for 14 days. The inhibition of C3HBA and C-755 tumors

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TABLE I

*Results of In Vitro Agar Tests with Fumagillin and Some of Its Moieties*

Agar plate tests	Inhibition expressed in mm.		
	1000 $\mu\text{g.}/\text{ml.}$	100 $\mu\text{g.}/\text{ml.}$	10 $\mu\text{g.}/\text{ml.}$
Decatetraenedioic acid	0	0	0
Fumagillin	23	14	0
Fumagillin alcohol-Ib	16	0	0
Dimethyl decatetraenedioate	0	0	0
Alcohol III of fumagillin	0	0	0
Dihydromonol F ( $\text{C}_{16}\text{H}_{20}\text{O}_4$ )	0	0	0

was determined on a wet weight basis. Inhibition of S-180 was determined on a basis of the average diameter of the tumor. Where the ascites were used, inhibition was determined by calculating the 90 per cent survival time of the animals. The per cent of extension of survival time of the experimental group is given.

Fumagillin was given to patients in the form of 10 mg. tablets. The fumagillin alcohol-I in sesame oil was administered by intramuscular injection.

## RESULTS

*Microbial Studies.* Preliminary screening of eight fumagillin alcohols containing a methoxy group was carried out against a number of test organisms. Inhibition of *B. subtilis* 6051 (wild type) was observed with alcohol-I (using disc assay–agar technique). A concentration of about 5  $\mu\text{g.}$  produced an inhibition zone of 25 ml. Dihydroalcohol-Ib showed slight inhibitory activity against this same organism at a concentration of about 25  $\mu\text{g.}$  (zone size 15 mm.). It was decided to test the alcohol-I and the dihydroalcohol-Ib in animals bearing tumors. The six other alcohols were inactive against *B. subtilis* 6051. All alcohols failed to inhibit *E. coli* B, *E. coli* W, and *A. aerogenes*.

The ability of fumagillin and some of its derivatives to inhibit dehydrogenase was tested on agar plates made with Ehrlich ascites diluted in Tyrode's solution. Area showing unreduced redox dye indicates that dehydrogenase has been in-

TABLE II

*Effect of Fumagillin Administration upon Tumors*

Tumor system	Results*	No. animals	Dosage, $\text{mg.}/\text{Kg.}/\text{day}$	Total no. injections	Per cent inhibition†	Per cent extension of survival time
S-180	$\pm$	10	8	14	33	
S-180	$\pm$	10	35	14	37	
Krebs-2 ascites	—	10	8	14		10
Krebs-2 ascites	—	10	35	14		—10
Ehrlich ascites	+	10	8	14		70
Ehrlich ascites	++	10	30	14		170
L-1210	—	10	10	14		15
L-1210	$\pm$	10	20	14		50
C-755	—	10	8	28	12	
C-755	+	10	20	28	67	
C-755	+	10	30	28	68	
C3HBA	+	10	30	28	54	

\* In the ascites tumors  $\pm$  indicates 20 to 50 per cent increase survival time of experimental group, and ++ indicates survival time exceeding 125 per cent. Per cent inhibition of solid tumors: no inhibition to 24 per cent, —; 25 to 49 per cent inhibition,  $\pm$ ; 50 to 74 per cent inhibition, +; 75 to 100 per cent inhibition, ++.

† Animals were given injections twice daily 24 hours after tumor transplantation.

TABLE III

*Effect of Fumagillin Alcohol-I Administration upon Tumors*

Tumor system	Results*	No. animals	Dosage, mg./Kg./day	Total no. injections†	Per cent inhibition	Per cent extension of survival time
Alcohol-I						
S-180	±	10	20	14	25	
S-180	—	9	35	14	11	
S-180	±	10	90	14	35	
S-180	±	30	125	14	30	
Krebs <sup>-2</sup> ascites	+	10	25	14		80
Krebs <sup>-2</sup> ascites	—	20	125	14		—3
Krebs <sup>-2</sup> ascites	—	10	250	14		15
Krebs <sup>-2</sup> ascites	—	10	550	14		—47
Ehrlich ascites	±	10	25	14		46
Ehrlich ascites	—	10	25	14		—7
Ehrlich ascites	—	10	125	14		9
Ehrlich ascites	—	10	250	14		—18
Ehrlich ascites	—	10	500	14		—20
L-1210	—	10	25	14		—27
C-755	±	8	8	28	40	
C-755	++	10	10	28	75	
C-755	+	30	20	28	70	
C-755	++	20	125	28	77	
C-755	++	10	250	28	78	
C3HBA	++	8	25	28	85	

\* In the ascites tumors ± indicates 20 to 50 per cent survival time of experimental group, and ++ indicates survival time exceeding 125 per cent. Per cent inhibition of solid tumors: No inhibition to 24 per cent, —; 25 to 49 per cent inhibition, ±; 50 to 74 per cent inhibition, +; 75 to 100 per cent inhibition, ++.

† Animals were given injections twice daily 24 hours after tumor transplantation.

hibited and that the tumor cells possibly have been killed. Table I shows that only fumagillin and fumagillin alcohol-I produce zones of inhibition. None of the compounds, including fumagillin, inhibited the growth of *Clostridium perfringens* growing on agar plates in Brewer anaerobic jars.

Fumagillin, its decatetraenedioic acid and its alcohols were tested on a series of yeast respiratory mutants and their parents. Since these mutants differ from their parents in their ability to utilize glucose by oxidation, they might differ in their response to various drugs. None of these compounds inhibited the growth of the yeast strains of their respiratory mutants.

*Animal Studies.* The LD<sub>50</sub> of fumagillin is approximately 800 mg./Kg. of body weight given subcutaneously in mice, although much higher doses are tolerated upon oral administration. Rats tolerated oral doses of 65 mg./Kg. for 18 days, although some growth abnormalities on doses higher than 6.5 mg./Kg. were observed. Daily oral doses of about 5 mg./Kg. often produced death in dogs after 9 or 10 days but were well tolerated by rabbits.

The LD<sub>50</sub> of fumagillin alcohol-I in Swiss mice is about 750 mg./Kg. administered intraperitoneally. Loss of motor control for one hour followed treatment. Injection of 600 mg./Kg. intraperitoneally caused no deaths and produced very little shock. Repeated injection of 500 mg./Kg. daily for four days resulted in an average loss of weight of 2.8 Gm. over five days.

Prior to receiving daily rations, two dogs were force fed 70 mg./Kg. in a no. 5 capsule for seven days. Both dogs were sacrificed on the tenth day, and each showed weight loss of 1 Kg. No gross or microscopic changes attributable to the fumagillin alcohol-I were found.

TABLE IV  
*Effect of Fumagillin on a Variety of Human Neoplasms*

Diagnosis	Dosage/ day/mg.	Total dosage, mg.	Complications		Effect on tumor
			Hematology	Other	
Epidermoid carcinoma esophagus	50 120 80	250 840 80	None	Nausea	None
Total		1170			
Metastatic carcinoma of neck, primary unknown	40 60 100	40 360 100	None	None	None
Total		500			
Adenocarcinoma of fundus	100 decreased to 50	600 750	None	Nausea	30 per cent decrease for 1 mo.
Total		1350			
Carcinoma of tongue with metastasis	150 200	150 3400	None	None	None
Total		3550			
Hypernephroma right kid- ney with pulmonary metastasis	100	1200	None	None	None
Recurrent leiomyosarcoma uterus	100	2200	None	Nausea, vomiting, diarrhea	None
Recurrent carcinoma of larynx, neck, and axilla	100	1500	None	Slight diarrhea	None
Carcinoma of gall bladder with liver metastasis	400	400	None	None	None
Carcinoma of colon with metastasis	100	1000	75,000 platelets	Anorexia, diarrhea	None
Carcinoma of lung and tibia	100	1500	75,000 platelets	None	None
Bronchogenic carcinoma with supraclavicular metastasis	100 50	400 50	Normal	None	None
Total		450			
Spindle-cell carcinoma of thyroid	40 60 120	40 360 120	22,000 platelets	None	None
Total		520			

Two more dogs were given fumagillin alcohol-I. One dog was given 200 mg./Kg. orally for seven days, and the second dog was given 300 mg./Kg. for seven days. The nonprotein nitrogen values of these 2 dogs were 35 to 39. The differential blood count of the 2 dogs showed scarcity of white cells, the white counts being 2000 and 6000/cu. mm. An autopsy was performed on both dogs on the tenth day. The dog that received the smaller amount per Kg. showed at autopsy moderate congestion of liver, gut, and kidney. The spleen was black and contracted, the pancreas bloody, the mucous membrane of the gums swollen, and there was some urine in the bladder. The dog that received 300 mg./Kg. had intestinal congestion, livid colored intestine, normal liver, empty bladder, contracted

spleen, no free fluid in the peritoneal cavity, and congested but macroscopically unchanged kidney.

Abbott Laboratories<sup>10</sup> found that intramuscular injection of fumagillin alcohol-I in rabbits (1 ml. of 10 per cent sesame oil solution) produced moderate irritation and necrosis in 24 hours which became more marked after 48 hours and 72 hours.

The results of the mouse tumor tests are given in tables II and III. By the criteria stated in table II fumagillin had no inhibitory effect on Krebs-<sup>2</sup> ascites, a  $\pm$  on S-180 and L-1210, a + effect on C-755 and C3HBA and a ++ effect on Ehrlich ascites. The greatest inhibition was usually accompanied by a 10 to 15 per cent weight loss of the host. Sugiura<sup>11</sup> has reported that injections of 25 mg./Kg./day of fumagillin may result in inhibition extending from + effect on S-180 to a 3 + effect on Ehrlich carcinoma. Table III presents a summary of the pertinent results obtained with the fumagillin-derived compounds on mouse tumors. The alcohol-I differs from its parent compound in being less toxic and producing greater inhibition of the mammary carcinoma and the adenocarcinoma. Also used were two series of 10 Bagg albino mice and controls which had skin tumors produced with 3-methylcholanthrene. All non-necrotic tumors were measured before and after treatment with 125 mg./Kg. of the alcohol-I. It was found that tumors less than 4 mm. disappeared completely, those 4 to 8 mm. were one half of the control size while tumors larger than 1 cm. appeared to be affected very little. The dihydro-alcohol-Iab, which has two more hydrogens than fumagillin but lacks the epoxide group, was ineffective as a tumor inhibitor at the concentrations used. Only small quantities of two other alcohols were available for limited testing and no encouraging results were obtained. Furthermore, neither the unsaturated side chain of fumagillin, decatetraenedioic, or the dimethyl derivative of the side chain inhibited any of the tumors at the concentrations used.

*Human Studies.* There are few general toxic reactions to fumagillin. Therapeutic doses in human beings have no known side effects upon liver, kidneys, or the blood forming organs. Large doses of fumagillin may result in anorexia, nausea, vomiting, abdominal cramps, and diarrhea. Lessening the dosage for a few days seems to control these symptoms. A few patients have been seen with a skin eruption of a vesicular type, involving the palmar and plantar surfaces.<sup>12</sup>

Twelve patients received fumagillin. One mg./Kg./day of fumagillin divided into three equal doses was administered orally. Total dosage given ranged from 400 to 3550 mg. The cases included spindle-cell carcinoma of thyroid, rectal leiomyosarcoma and several carcinomas with and without metastases. No effect was observed on any tumor. Complications from the treatment were either nonexistent or very mild. Following treatment, 3 patients had depressed platelet counts and 3 patients had diarrhea, 2 of whom had either accompanying anorexia or nausea and vomiting (table IV).

Thus far alcohol-I has been given to 2 patients. Intramuscular injections of 100 mg. in sesame oil/day are being given for three days followed by a five day observation period between a second course of treatment. One patient with a diagnosis of malignant melanoma was given two courses of treatment of fumagillin alcohol-I. No improvements or complications were attributed to fumagillin alcohol-I and death of the patient was unrelated to therapy.

#### CONCLUSION

In tests with fumagillin and several compounds derived from it, fumagillin and alcohol-I showed the most activity in the experimental systems. Fumagillin had no

cancer therapeutic effect on the series of 12 patients. Fumagillin alcohol-I is undergoing further clinical trial.

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#### BIBLIOGRAPHY

1. McCOWEN, M. C.; CALLENDER, M. E., AND LAWLIS, J. F., JR.: Fumagillin (H-3) a new antibiotic with amebicidal properties<sup>1,2,3</sup>, *Science* 113:202, 1951.
2. HANSON, F. R., AND EBLE, T. E.: An antiphage agent isolated from *aspergillus* sp., *J. Bact.* 58:527, 1949.
3. ROSS, J. M.; TARBELL, D. S.; LOVETT, W. E., AND CROSS, A. D.: The chemistry of Fumagillin. IV. The presence of an epoxide grouping and other observations on the nature of the oxygen functions, *J. Am. Chem. Soc.* 78:4675, 1956.
4. LANDQUIST, J. K.: Some degradation products of fumagillin, *J. Chem. Soc.* 78:4237, 1956.
5. SCHENCK, J. R.; HARGIE, J. P.; TARBELL, D. S., AND HOFFMAN, P. J.: Decatetraenedioic acid, a fumagillin degradation product, *J. Am. Chem. Soc.* 75:2274, 1953.
6. TARBELL, D. S.; HOFFMAN, P.; ALKAZIMI, H. R.; PAGE, G. A.; ROSS, J. M.; VOGT, H. R., AND WARGOTZ, B.: The chemistry of Fumagillin, III, *J. Am. Chem. Soc.* 77:5610, 1955.
7. TARBELL, D. S., AND VARINO, F. H. L.: Unpublished work, University of Rochester.
8. DiPAOLO, J. A., AND MOORE, G. E.: An evaluation of ascites tumor cell plating for screening chemotherapeutic agents, *Antib. & Chemo.* 7:465, 1957.
9. DiPAOLO, J. A., AND ROSENFELD, R.: The use of anaerobes and respiratory deficient yeast mutants as a screening procedure for potential tumor inhibiting agents, *Cancer Research*. In press.
10. ANON.: Personal communication from Abbott Laboratories.
11. SUGIURA, K.: Merits of ascites tumors for screening, Part I, *Ann. New York Acad. Sc.* 63:962, 1956.
12. ANON.: Personal communication to Abbott Laboratories.

# Origin of a New Antitumor Agent, Streptovitacin

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The availability of antibiotics as sources of new antitumor agents has been recognized for some time. As early as 1941 Waksman isolated an actinomycin that had a selective cytotoxic activity in certain tumors; subsequent studies of the antitumor activity of the actinomycetes by others resulted in the isolation of actinomycin C, a clinical agent of limited usefulness.<sup>1,3</sup> More recently Waksman has isolated another agent of some potency, now identified as actinomycin D.<sup>4</sup> Azaserine and puromycin are other agents isolated from culture filtrates of soil organisms.<sup>5,6</sup> Thus, it appears that antibiotic filtrates may be good sources of effective antitumor agents and many centers are now engaged in screening antibiotics and their derivatives.

This laboratory has had a strong interest in antibiotics as potential antitumor agents and has evaluated a number of such agents during the past few years.<sup>7,9</sup> In the course of these studies a commercially available antibiotic preparation was obtained for study in the program. It was cycloheximide,\* a product of a strain of *Streptomyces griseus*. However, it gave variable results in the antitumor assays. Because it is felt here that borderline results in antitumor chemotherapy screening deserve further study where possible, the remaining beer solids of the fermentation were obtained and it was found that the preparation gave a significant inhibition of the tumors. The purpose of this report is to present the data that initiated the program resulting in the isolation of a new antitumor agent, which is the subject of the series of papers that follow.

## EXPERIMENTAL

*Methods.* The mouse Crocker sarcoma and the mouse RC carcinoma are the basic tests used in this laboratory.<sup>3</sup> Under aseptic conditions, a 7 day old tumor was divided into fragments obtained from non-necrotic areas. These measured about 1.5 mm. in all dimensions and were implanted by trocar subcutaneously into the axilla of healthy mice.

The mice used were females, 18 to 22 Gm. in weight. White swiss females were used for the sarcoma 180 and DBA female mice were used for the RC carcinoma. The mice were maintained on an ad libitum diet of Purina laboratory chow, barley, and water.

The commercial solution of antibiotic, cycloheximide, was dissolved in sterile saline solution and the beers were suspended in 1 or 5 per cent gum acacia. They were given in the doses stated, in a volume of 0.5 ml. administered intraperitoneally beginning 24 hours after tumor implantation. The preparations were given once daily for seven consecutive days to the mice bearing the tumors.

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\* The trade name of The Upjohn Company for cycloheximide is Acti-dione.

TABLE I  
*Effect of a Preparation of Streptomyces Griseus Beer Solids on Tumor Growth*

Dose, mg./Kg./day	Route	No. tests	No. deaths	Per cent inhibition of tumor	
				Average	Range
<i>Sarcoma 180</i>					
250	Intraperitoneal	1	2/5	43	
300	Intraperitoneal	3	2/15	51	36-72
250	Oral	1	0/5	20	
500	Oral	2	3/12	26	20-33
<i>RC Carcinoma</i>					
250	Intraperitoneal	2	1/10	41	33-50

At the conclusion of the injection period, the tumors were measured in two diameters by calipers, the average tumor size was calculated and the treated and control groups were compared. Routinely comparison tests with anti-neoplastic agents of known significant potency were undertaken. In different assays either triethylene melamine (TEM) or amethopterin were used.

#### RESULTS

*Effect of Cycloheximide on Sarcoma 180.* When given in doses of 30 to 60 mg./Kg./day, the average inhibition of sarcoma 180 was 25 per cent of the control tumors in four tests with a range of 10 to 52 per cent. Although this degree of activity is not usually considered to be more than of slight interest, it was felt desirable to proceed with additional studies and the remaining beer solids of this fermentation were then evaluated.

*Effect of the Beer Solids of the Streptomyces Fermentation on Mouse Tumors.* The beer solids from the *Streptomyces* fermentation described were suspended in 1 or 5 per cent gum acacia and given both intraperitoneally and by stomach tube to mice with implants of sarcoma 180. A summary of the results is given in table I. At a level of 300 mg./Kg./day, the preparation induced an average reduction of 51 per cent of the tumors in the treated mice from that of the control mice. It was apparent that the material possessed antitumor activity when given

TABLE II  
*Effect of Fractions of Treated Streptomyces Griseus Beer Solids on Tumor Growth*

Sarcoma 180 sample no.	Dose	Route	No. tests	No. deaths	Per cent inhibition of tumor	
					Average	Range
3729	150	Intraperitoneal	2	0/10	29	26-33
	300	Oral	1	0/5	18	
3730	100	Intraperitoneal	2	1/10	30	30-31
	200	Oral	1	2/5	27	
3731	75	Intraperitoneal	2	0/10	3	2-5
	150	Oral	1	2/5	9	
3732	175	Intraperitoneal	2	0/10	46	42-51
	350	Oral	2	2/10	20	
3733	200	Intraperitoneal	3	4/15	27	20-33
	400	Oral	2	0/10	19	
3734	200	Intraperitoneal	3	4/15	31	16-48
	400	Oral	2	0/10	22	

orally. A dose of 500 mg./Kg./day given by stomach tube produced a reduction of tumor size of 26 per cent from control mice. Mice with RC carcinoma were given 250 mg./Kg./day of the beer suspension. On the eighth tumor day, the size of the tumors of the treated mice was 41 per cent of their controls.

*Effect of Fractions of a Strain of Streptomyces griseus on Mouse Tumors.* Fractionation and microbiological assay procedures and the chemical properties of streptovitacin A will be reported in subsequent papers in this series.<sup>10-12</sup> Evans et al separately described their tumor assay procedures, which facilitated the isolation of a purified preparation.<sup>13</sup> A sampling of the early fractionations tested against our tumors is given in table II. It will be seen that although there was some variation, the majority of the specimens possessed significant antitumor activity.

#### DISCUSSION

The observation that cycloheximide, a soluble fermentation product of *S. griseus*, produced a mild but variable degree of tumor inhibition provoked an examination of products related to the specific material. In the next step of this study the residual solids of the fermentation were evaluated. When suspensions of this material were given both by the parenteral and oral routes to mice with sarcoma 180 and RC breast adenocarcinoma, significant inhibition was obtained. Fractionation procedures were undertaken and six sets of the early replicate products of the *Streptomyces* fermentation were evaluated. In general, these also possessed significant antitumor activity. This series of observations initiated the fractionation and characterization procedures which resulted in the isolation of a crystalline agent, streptovitacin A, a new antitumor principle, the subject of the following reports.

#### SUMMARY

1. When cycloheximide, a soluble fermentation product of *Streptomyces griseus*, was given to mice with sarcoma 180, a moderate but variable degree of tumor inhibition was obtained.

2. A suspension of the residual beer solids of the same fermentation was given intraperitoneally at levels of 300 mg./Kg./day. The average reduction of sarcoma 180 tumors was 51 per cent of the controls. When 250 mg./Kg./day was given to mice with RC carcinoma the average reduction of tumors was 41 per cent of the controls. Antitumor activity was also obtained when the beer solids were given by stomach tube.

3. Six sets of replicate products were obtained in fractionation of the *S. griseus* fermentation and these gave an average reduction in the size of sarcoma 180 of 28 per cent of control tumors.

4. These observations initiated the fractionation and characterization procedures which resulted in the isolation of a new antitumor principle, streptovitacin A.

#### BIBLIOGRAPHY

1. HACKMANN, C.: Experimentelle untersuchungen über die wirkung von actinomycin C (HBF 386) bei bösartigen geschwülsten, Ztschr. f. Krebsforsch. 58:607, 1952.
2. SCHULTE, G.: Erfahrungen mit neuen cytotatischen mitteln bei hämoblastosen und carcinomen und die abgrenzung ihrer wirkungen gegen röntgentherapie, Ztschr. f. Krebsforsch. 58:500, 1952.
3. FIELD, J. B.; COSTA, F.; BORYCZKA, A., AND SEKELY, L.: Experimental evaluation of the anticarcinogenic activity of a new antibiotic, actinomycin C. In: Antibiotics Annual 1954-1955, New York, Medical Encyclopedia, Inc., 1955, p. 842.

4. MANAKER, R. A.; GREGORY, F. J.; VINING, L. C., AND WAKSMAN, S. A.: Actinomycin III. The production and properties of a new actinomycin. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, p. 853.
5. ELLISON, R. R.; KARNOFSKY, D. A.; STERNBERG, S. S.; MURPHY, M. L., AND BURCHENAL, J. H.: Clinical trials of o-diazo-acetyl-L-serine (Azaserine) in neoplastic disease, *Cancer* 7:801, 1954.
6. WRIGHT, J. C.; DOLGOLPOL, V.; LOGAN, M.; PRIGOT, A., AND WRIGHT, L. T.: Clinical evaluation of puromycin on human neoplastic disease, *Proc. Am. A. Cancer Research* 2:55, 1955.
7. FIELD, J. B.: The carcinolytic effect of Actinomycin C. A report of clinical studies, *California Med.* 86:305, 1957.
8. FIELD, J. B.; FILLER, D. A.; BASCOY, L. T.; COSTA, F., AND BORYCZKA, A.: Anticarcinogenic activity of several antibiotics, *Western Soc. for Clinical Research*, Carmel, Cal. Jan. 27-28, 1956.
9. FIELD, J. B.; FILLER, D. A.; BASCOY, L. T.; COSTA, F., AND BORYCZKA, A.: Antibiotics as sources of anticarcinogens, *Federation Proc.* 15:816, 1956.
10. EBLE, T. E.; BERGY, M. E.; LARGE, C. M.; HERR, R. R., AND JACKSON, W. G.: Isolation and purification of streptovitacins A and B. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 555.
11. SOKOLSKI, W. T.; EILERS, N. J.; SAVAGE, G. M.: Paper chromatography and microbiologic assay of the streptovitacins. *Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 551.
12. HERR, R. R.: Structure studies on streptovitacins A and B. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 560.
13. EVANS, J. S.; MENGEL, G. D.; CERU, J., AND JOHNSTON, R. L.: Biological studies on streptovitamin A, a new antitumor agent. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 565.

# Paper Chromatography and Microbiological Assay of the Streptovitacins

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The streptovitacins are a mixture of closely related components found in fermentations of a cycloheximide\* producing strain of *Streptomyces griseus*. Biological studies, including antitumor activity of streptovitamin A are described by Field et al.,<sup>1</sup> Evans et al.,<sup>2</sup> and Field et al.<sup>3</sup> Isolation and structure studies of streptovitacins A and B are presented by Eble et al.<sup>4</sup> and Herr.<sup>5</sup> This paper describes paper chromatographic systems for resolution of the streptovitamin mixture and microbiological assays for streptovitacins A and B.

## METHODS AND RESULTS

*Paper Chromatography.* A crude preparation, containing 5 per cent each of streptovitacins A and B, 0.1 per cent cycloheximide and undetermined amounts of streptovitacins C, D, and E, was paper chromatographed with several solvent systems. Six hundred  $\mu\text{g.}$  were applied to Schleicher and Schuell 589 (blue ribbon special) paper for each system. After the strips were developed and dried, they were bioautographed on test trays with *Saccharomyces pastorianus* ATCC 2366. The trays were incubated for 18 hours with the strips left on. The locations of zones of inhibition indicating the position of the different components are shown in figure 1. The following test systems were used at 25 C.: I. Water-saturated ethyl acetate; paper pre-impregnated with 0.1 *M* phosphate solution at pH 4.0. II. Upper phase of benzene:methanol:water, 1:1:2 (v/v). III. Butanol:water, 84:16 (v/v).

For systems I and II, strips were equilibrated above both phases of the solvent mixture for 16 hours and developed for six hours. For system III, strips were developed for 16 hours without pre-equilibration.

System I is the test system used in the assay for streptovitacins A and B. The  $R_f$  values in this system are 0.38 for A and 0.45 for B.

## ASSAY FOR STREPTOVITACINS A AND B

The assay methods for streptovitacins A and B use the same principles as other paper chromatographic assays.<sup>6-8</sup> The biological test system is *S. pastorianus* ATCC 2366 in medium containing 1 per cent Cerelose, 0.25 per cent yeast extract, 0.1 per cent monobasic potassium phosphate, and 1.5 per cent agar.

Schleicher and Schuell 589 paper is cut into 6 by 22½ inch sheets. The sheets are impregnated with 0.1 *M* pH 4.0 phosphate solution (monobasic potassium phosphate and phosphoric acid) and dried. The impregnated sheets are then cut into ½ by 22½ inch strips.

A single standard solution is prepared in water such that each ml. contains 2 mg. of streptovitamin A and 2 mg. of streptovitamin B. The standard solution is applied to four strips with doses of 5, 10, 20, and 40  $\mu\text{l.}$ , giving a standard curve

\* The trade name of The Upjohn Company for cycloheximide is Acti-dione.

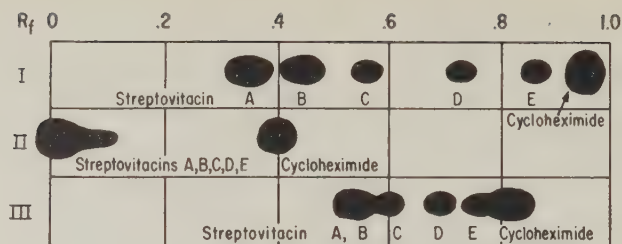


FIG. 1. Illustrated is a paper chromatography of *Saccharomyces pastorianus*, active components in a cycloheximide fermentation.

range of 10 to 80  $\mu\text{g.}$  per component. Test solutions are applied with volumes estimated to be within the standard range.

The strips are equilibrated for 16 hours above both phases of an ethyl acetate-water mixture, developed for six hours with the upper phase, and dried. Four standard strips and eight test strips are plated on each 8 by 20 inch *S. pastorianus* tray as shown in figure 2. The 8 inch segment of each strip that is in contact with the agar is the portion between 2 and 10 inches from the origin. The trays are incubated for 16 hours at 37 C. with the strips left on. The strips are removed and potencies of test solutions are estimated from standard curves plotted as zone width vs. logarithmic dose.

Figure 3 shows standard dose-response curves. The standard error of the assay method was estimated to be 16 per cent for each component.

For the assay of fermentation beers and preparations that contain low concentrations of streptovitamins A and B and a high concentration of cycloheximide, a modified method is used. The beers are freeze-dried and reconstituted to one tenth or less of the original volume. The concentrated solutions are applied to strips as volumes of 10 to 100  $\mu\text{l.}$  and the strips are developed for 72 hours with system II. This development removes cycloheximide and other impurities while streptovitamins A and B remain at the origin. The strips are then dried and redeveloped with system I.

#### ANTITRICHOMONAL ASSAY

The first paper chromatography pattern for streptovitamin was obtained by bioautographing developed paper strips against *Trichomonas vaginalis*. The method used was an adaptation of a microbiologic assay for streptovitamin A, developed by Michaels of these laboratories.

Whatman no. 1 paper strips,  $\frac{1}{4}$  by 22 $\frac{1}{2}$  inches, were used in all systems. One hundred and twenty  $\mu\text{g.}$  of the crude streptovitamin preparation were applied to each strip and the strips were developed with the indicated systems and dried. Each

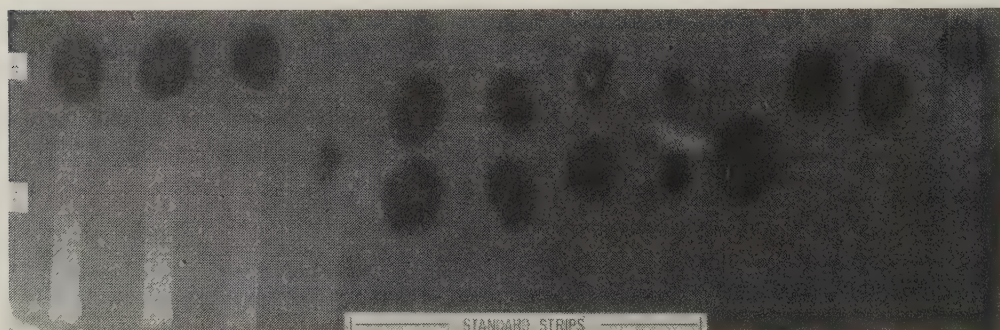


FIG. 2. Bicautograms of streptovitamins A and B on an *Saccharomyces pastorianus* tray are shown, with solvent flow from bottom to top.

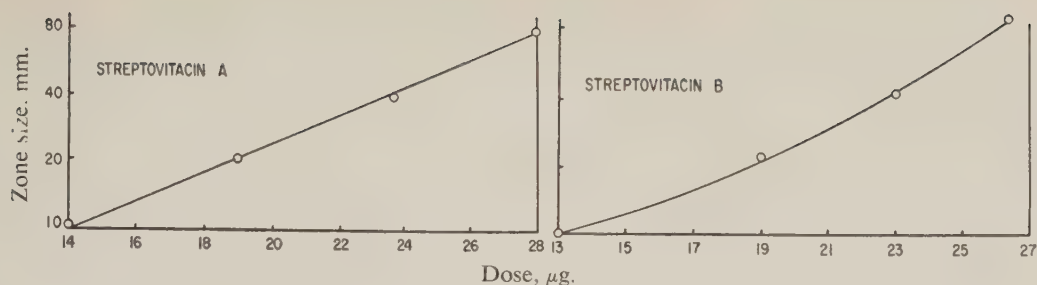


FIG. 3. Standard dose response curves for streptovitacins A and B are given.

strip was cut into 4 cm. sections and the sections were exposed to ethylene oxide vapors for one hour. Each section was dropped into a tube containing 10 ml. of *T. vaginalis* culture. The medium was fluid thioglycollate medium (BBL) with 5 per cent horse serum (Difco) and novobiocin at 10  $\mu\text{g.}/\text{ml.}$  The novobiocin was added to suppress bacterial contamination. The broth was seeded with a 24 hour culture to a final concentration of 10,000 cells/ml. The tubes were incubated for 48 hours at 37 C. After incubation, the cells were counted on a hemacytometer. Low counts were an indication of antitrichomonal activity.

In addition to systems II and III described the following systems were used: IV. Butanol:water, 84:16 plus 0.25 per cent (w/v) *p*-toluenesulfonic acid. V. Butanol:acetic acid:water, 2:1:1 (v/v). VI. Butanol:water, 84:16, 2 ml. of piperidine added to 98 ml. of butanol-water mixture. VII. Water:butanol, 96:4 (v/v). VIII. Water:butanol, 96:4 plus 0.25 per cent (w/v) *p*-toluenesulfonic acid. IX. 0.01 *N* ammonium hydroxide saturated with methyl isobutyl ketone. X. 0.1 *M* phosphate buffer pH 7.0.

Table I shows the trichomonal counts for the active strip sections. Control tubes without strip sections had counts of 87 and 95 cells/0.0001 ml. All cultures were examined microscopically and if the population appeared to be as high as the control, counts were not made. These results are designated as C (count resembling that of control cultures). Counts of 50 or less organisms/0.0001 ml. were recorded. Subsequent experience indicated that slight inhibitory effects are encountered from solvent residue or impurities from the paper. As a result, only those cell counts of 20 or less were interpretable as indicating activity.

The sections showing trichomonal counts of 20 or less are in bold type to emphasize the active sections. No activity was obtained from sections of the strip developed with system VI due to the inactivation of streptovitamin during

TABLE I  
*Bioautogram of Developed Paper Strips with T. vaginalis*

System	Organism count per 0.0001 ml., strip section									
	1	2	3	4	5	6	7	8	9	10
II	<b>5</b>	C	C	C	C	C	C	C	C	C
III	C	C	C	<b>20</b>	<b>5</b>	C	C	C	C	C
IV	C	C	C	C	<b>18</b>	34	C	C	C	C
V	C	C	C	C	49	31	<b>7</b>	44	C	C
VI	C	C	C	C	C	C	C	C	C	C
VII	C	C	C	C	C	C	C	<b>8</b>	C	C
VIII	42	46	C	40	C	39	C	<b>2</b>	30	C
IX	41	40	49	31	45	43	31	<b>7</b>	39	C
X	C	48	40	28	C	C	C	<b>4</b>	28	49

development with this alkaline system. The activities indicate the locations of both streptovitacins A and B, since these entities are not separated by any of the nine systems.

#### DISCUSSION

Resolution of streptovitacins A and B can actually be accomplished by system I without pre-impregnation of the strips. Impregnation with 0.1 *M* phosphate solutions was found to give superior resolution and pH 4.0 impregnation was finally selected for optimal separation.

The minimum dose of streptovitacins A or B for an *S. pastorianus* response is approximately 100 times that for cycloheximide, or 5  $\mu\text{g.}$  for either of the two streptovitamin components and 0.05  $\mu\text{g.}$  for cycloheximide. With a maximum volume of 100  $\mu\text{l.}$  applied to a strip, the lower sensitivity limit in the *S. pastorianus* assay is 50  $\mu\text{g./ml.}$  for streptovitacins A or B.

*T. vaginalis* is much more sensitive to streptovitamin A than is *S. pastorianus*. However, the *T. vaginalis* method is not reliable for test preparations that contain cycloheximide, streptovitamin B, or other antitrichomonal agents. The concentrations in  $\mu\text{g./ml.}$  which inhibit the organism to 50 per cent of the maximum growth ( $\text{IC}_{50}$ ) are: 0.04 for streptovitamin A, 0.4 for streptovitamin B, and 0.1 for cycloheximide.

The sensitivity of *T. vaginalis* to streptovitamin A is such that the organism might be used in a blood assay procedure. Preliminary studies indicate that human serum can replace horse serum in the medium for supporting growth of *T. vaginalis*. By adding 0.5 ml. of test serum to 9.5 ml. of culture, 0.8  $\mu\text{g.}$  of streptovitamin A per ml. of test serum may be detected. The use of a higher ratio of serum to broth might increase the sensitivity of the method.

#### ACKNOWLEDGMENT

The authors are indebted to Mr. C. M. Large and Drs. T. E. Eble and R. R. Herr for the streptovitamin standard preparations, to Mrs. L. J. Gregory for technical assistance, and to Dr. O. S. Carpenter for the statistical analyses.

#### BIBLIOGRAPHY

1. FIELD, J. B.; COSTA, F., AND BORYCZKA, A.: Origin of a new antitumor agent, streptovitamin. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 547.
2. EVANS, J. S.; MENGEL, C. D.; CERU, J., AND JOHNSTON, R. L.: Biological studies on streptovitamin A, a new antitumor agent. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 565.
3. FIELD, J. B.; MIRALES, A.; PACHL, H. R.; BASCOY, L.; CANO, L., AND BULLOCH, W. K.: Experimental evaluation of a new antitumor agent, streptovitamin A. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 572.
4. EBLE, T. E.; BERGY, M. E.; LARGE, C. M.; HERR, R. R., AND JACKSON, W. G.: Isolation, purification, and properties of streptovitamins A and B. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 555.
5. HERR, R. R.: Structure studies on streptovitamins A and B. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 560.
6. KLUENER, R. G.: A paper chromatography method for the quantitative estimation of penicillin entities, *J. Bact.* 57:101-109, 1949.
7. KARNOVSKY, M. L., AND JOHNSON, M. J.: Filter paper chromatography of penicillin broths, *Anal. Chem.* 21:1125-1132, 1949.
8. SOKOLSKI, W. T.; EILERS, N. J., AND SIMINOFF, P.: Paper chromatography and assay of components in streptovaricin. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 119.

# Isolation, Purification, and Properties of Streptovitacins A and B

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This paper concerns the isolation and purification of the antitumor agent, streptovitamin A, and related substances from a *Streptomyces griseus* fermentation broth. Some characterizing physical data, solubility, and stability are described. Other papers in this series cover biological activity,<sup>1-3</sup> assay,<sup>4</sup> and structural studies.<sup>5</sup>

## ISOLATION

Crystalline streptovitamin A can be isolated by the following procedure: The whole broth is acidified to approximately pH 3.5 and filtered. The less polar constituents, including cycloheximide,\* are removed from the filtrate by extraction with chloroform or methylene chloride, and the streptovitacins are adsorbed from the aqueous solution onto carbon. Elution from carbon is accomplished with acidified aqueous acetone. The streptovitacins in the aqueous concentrate after vacuum distillation are adsorbed onto Permutit DR and desorbed by gradient elution from the resin with water to 50 per cent ethanol. The peak fractions from gradient elution are pooled, concentrated, and fractionated further by partition chromatography on Dicalite columns. A mixture of streptovitacins A and B can be crystallized from pools of chromatographic fractions following concentration, transfer to dry 1-butanol, and addition of cyclohexane.

Pure streptovitamin A can be obtained by repeated fractional crystallization, especially from acetonitrile or 1-butanol-ether, or by partition chromatography using the system ethyl acetate, cyclohexane, and pH 5 McIlvaine's buffer (7:1:8), or by countercurrent distribution in the system *n*-amyl alcohol, iso-amyl alcohol, and water (12:17:29). These steps afford yields and potencies of streptovitamin A, which are summarized graphically in figure 1. Crystalline streptovitamin A obtained in this manner is approximately 95 per cent pure by countercurrent distribution analysis. This is diagrammed in figure 2.

Streptovitamin B can be isolated from streptovitamin A mother liquors, or by the partition chromatography described, followed by evaporation of appropriate fractions, freeze-drying, crystallization from 1-butanol and cyclohexane, and recrystallization from acetonitrile and ether.

## CHARACTERIZATION

The biological properties of the crude streptovitamin mixture differentiated it from known antibiotics and antitumor agents. It was devoid of antibacterial activity against numerous organisms but possessed limited antifungal activity, and significant activity against the parasite, *Trichomonas vaginalis*. Although its activity against the yeast, *Saccharomyces pastorianus*, was eventually utilized for the refinement of purification procedures, the original isolation of streptovitamin A was accomplished with the aid of the Walker adenocarcinoma assay in rats. The crude

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\* The trade name of The Upjohn Company for cycloheximide is Acti-dione.

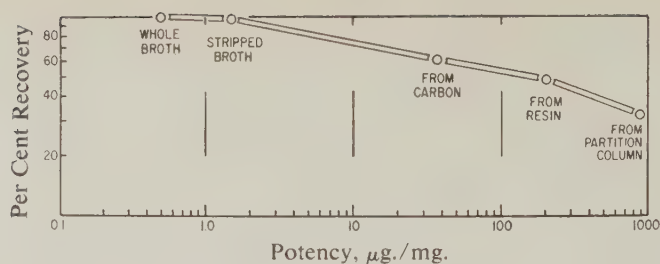


FIG. 1. Streptovitacin A isolation.

material was hygroscopic, very soluble in water, but insoluble in all but the most polar organic solvents. Crude material was neutral, and, in general, had properties suggestive of a carbohydrate nature.

Streptovitacin A exists in two crystalline modifications, orthorhombic (form I) and monoclinic (form II). Neither is hygroscopic. The two forms are readily distinguishable by microscopic examination, but, presumably because of facile inter-conversion, their infrared spectra and roentgen-ray powder diffraction patterns are identical. Refractive index measurements show values for the two forms that are not significantly different. The differences in optic axial angle, measured directly on each form, are conclusive. Also conclusive is the difference in crystal system: the monoclinic angle for form II is  $59^\circ$ , usually measured with an accuracy of one degree. The corresponding angle, by definition, for form I is  $90^\circ$ . Fusion studies show solid-solid transformation from the orthorhombic to the monoclinic form with unagitated unirradiated crystals at  $147^\circ\text{C}$ . The monoclinic form then melts at  $156$  to  $161^\circ\text{C}$ .

Molecular weight determinations on streptovitacin A by isothermal distillation in acetone gave an average value of 285. The analytical data on streptovitacins A and B (which are isomers) indicate the molecular formula  $\text{C}_{15}\text{H}_{23}\text{NO}_5$  (molecular weight, 297). The analytical data are presented later.

Crystalline streptovitacin A is soluble in water, less soluble in polar organic solvents, and essentially insoluble in nonpolar solvents. The data are summarized in table I.

Dry streptovitacin A stored at  $70^\circ\text{C}$ . showed no detectable degradation after 60 days. Deionized water solutions in the range 0.05 to 0.5 mg./ml. and initial pH 6.7

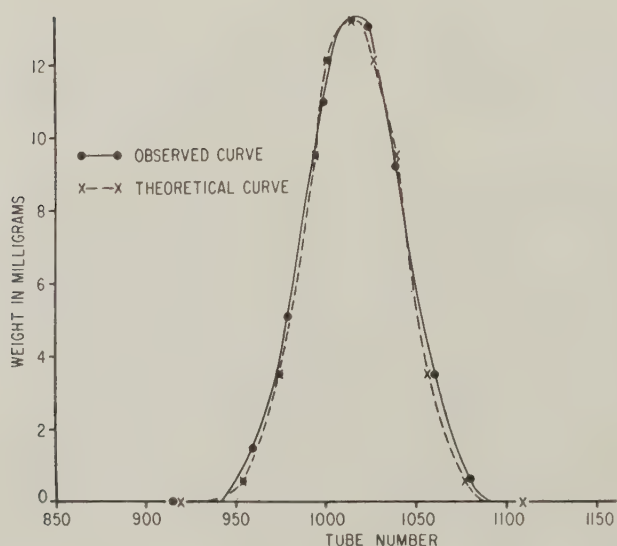


FIG. 2. The countercurrent distribution of streptovitacin A, 2500 transfers.

TABLE I  
*Solubility of Streptovitacin A at 25 C.*

	mg./ml.
Water	>300
Ethanol (95 per cent)	150
Acetonitrile	45
1-Butanol	20
Ethyl acetate	13

assayed 50 to 70 per cent of original potency after five weeks at 38 C., and 100 per cent of original potency after five weeks at 25 C. It is relatively stable in aqueous acid solutions but degrades rapidly in alkaline solution. It shows no titratable groups in aqueous solution but hydrolysis at pH 12 liberates an acidic function with a  $pK_a'$  of 4.8.

Although streptovitacin A shows little rotation at the sodium D line, it is optically active as shown by the rotatory dispersion curve (in dioxane) of figure 3. The lack of measurable rotation in water above 280  $m\mu$  has been interpreted as reaction of the optically active chromophore with water.

Ultraviolet absorption studies on streptovitacins A or B show end absorption only. The infrared absorption spectrum of streptovitacin A is shown in figure 4. The spectrum in mineral oil suspension shows identifying absorptions at the following frequencies: 3480, 3380, 3180, 3070 (OH/NH), 1715, 1682, 1655 (C = O, C = N), 1310 (shoulder), 1275, 1235, 1230, 1150, 1140, 1118, 1100 (shoulder), 1075 and 1030  $cm.^{-1}$ . The infrared spectrum of streptovitacin B (fig. 5) shows identifying absorptions at the following frequencies: 3560, 3460, 3150, 3050 (OH/NH), 1715, 1697, 1677 (C = O, C = N), 1407, 1310, 1285, 1275 (shoulder), 1227, 1170, 1150, 1130, 1108, 1078, 1066, 1050 and 1033  $cm.^{-1}$ .

Further physical characterizations for the streptovitacins are provided by paper chromatographic analysis, the details of which were presented in an earlier paper in this series.<sup>4</sup>

#### EXPERIMENTAL STUDIES

*Isolation from Culture Broth.* Five thousand liters of a culture broth of the streptovitacins was adjusted to pH 3.5, heated to 60 C. for 10 minutes and cooled to 30 C. The broth was then mixed with 200 Kg. of Dicalite 4200 and filtered; the filtrate was extracted with methylene chloride. The aqueous phase, containing approximately 132 Gm. of streptovitacin A, was stripped of solvent and stirred with 180 Kg. of Darco G-60. The carbon was collected by filtration and statically eluted with 1000 liters of 85 per cent acetone. The eluate was concentrated and freeze-dried to afford 2.2 Kg. of material containing 81 Gm. of streptovitacin A (potency 37  $\mu g./mg.$ , yield 62 per cent).

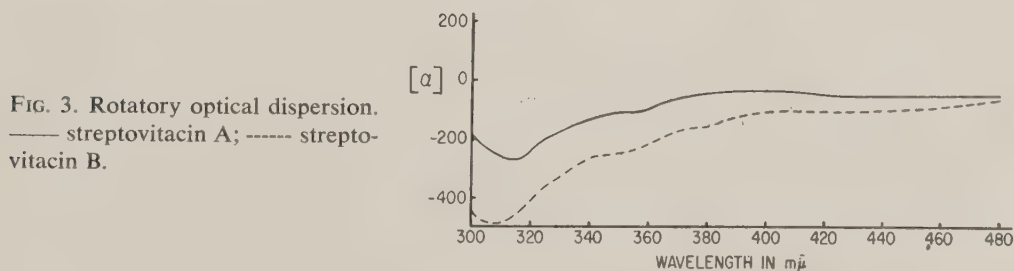


FIG. 3. Rotatory optical dispersion.  
— streptovitacin A; ---- streptovitacin B.

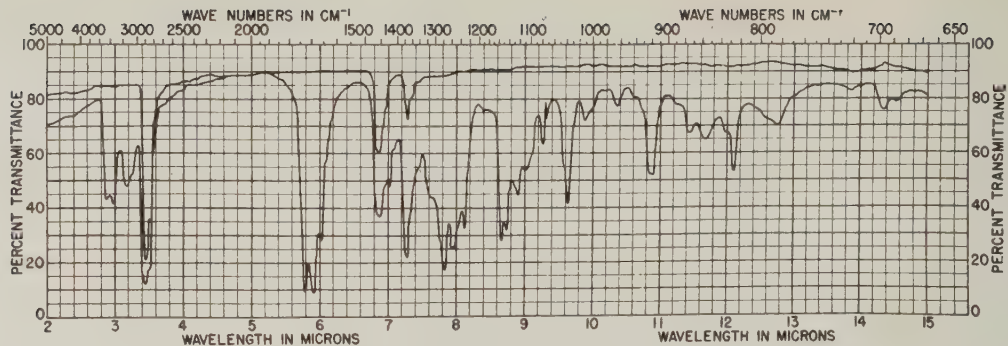


FIG. 4. Infrared absorption spectrum of streptovitamin A, mineral oil mull.

*Resolution on Permutit DR.* One and 0.1 Kg. of material obtained as above described was dissolved in 5.5 liters of water, placed on a 37 liter bed of regenerated Permutit DR in a 6 inch column, 6 feet high, and washed with 100 liters of water at a rate of 700 ml. per minute. The column was eluted by means of a linear concentration gradient<sup>6</sup> of water to 50 per cent alcohol using a total of 55.1 of 50 per cent 3A alcohol and 55 l. of water (followed by 16 l. of 50 per cent 3A alcohol) at a rate of approximately 300 ml./minute. Four liter fractions were collected and analyzed for solids and bio-activity. The most active material was found on the slope of the ascending weight curve. These fractions, numbers 1 to 32, gave 182 Gm. of product containing 20 Gm. of streptovitamin A (potency 110  $\mu$ g./mg., yield 49 per cent).

*Partition Chromatography.* The solvent system consisted of 7 parts of ethyl acetate, 1 part of cyclohexane, and 8 parts of pH 5.0 McIlvaine's buffer. A slurry of 6 Kg. of unwashed Dicalite 4200, 2.4 liters of lower phase and a minimum amount of upper phase was poured into a 6 inch column and packed with 5 lb./sq. in. air pressure to a height of approximately 50 inches. A charge of 475 Gm. of material obtained from Permutit DR columns, containing approximately 52 Gm. of streptovitamin A, was dissolved in 363 ml. of lower phase. The solution was mixed with 725 Gm. of Dicalite and a minimum amount of upper phase, and placed on the column.

The column was eluted with upper phase at a rate of 60 ml./minute. It was monitored by spotting fractions on *S. pastorianus* agar culture. The streptovitamins were eluted from the column after about three to four liquid holdups. A fraction containing approximately 45 Gm. of streptovitamin A was concentrated by azeotropic distillation to an aqueous solution which was freeze-dried. The product, after crystal-

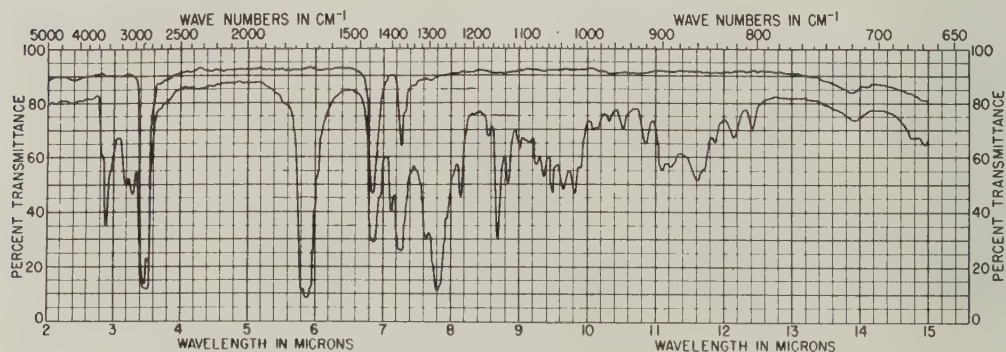


FIG. 5. Infrared absorption spectrum of streptovitamin B, mineral oil mull.

lization from acetonitrile, weighed 39 Gm. and assayed about 900  $\mu\text{g.}$  of streptovitamin A per mg. (yield 67 per cent).

*Analytical Results for Streptovitamin A.* A sample of crystalline streptovitamin A was recrystallized successively from acetonitrile, 1-butanol and ether, ethyl acetate, and twice again from acetonitrile. The recrystallized sample melted at 156—159 C.

ANALYTICAL. Calculated for  $\text{C}_{15}\text{H}_{23}\text{NO}_5$ : C, 60.58; H, 7.80; N, 4.71; O, 26.91;  $2(\text{C}-\text{CH}_3)$ , 10.1. Found: C, 60.71; H, 8.05; N, 4.73; O, 27.00;  $\text{C}-\text{CH}_3$ , 7.7; molecular weight by isothermal distillation in acetone gave the values 283, 284, and 287.

*Analytical Results for Streptovitamin B.* A sample of streptovitamin B was crystallized from 1-butanol-cyclohexane and recrystallized from acetonitrile-ether. The recrystallized sample melted at 124—128 C.

ANALYTICAL. Calculated for  $\text{C}_{15}\text{H}_{23}\text{NO}_5$ : C, 60.58; H, 7.80; N, 4.71; O, 26.91;  $2(\text{C}-\text{CH}_3)$ , 10.1. Found: C, 60.66; H, 7.87; N, 4.71; O, 27.57;  $\text{C}-\text{CH}_3$ , 8.6.

*Crystallographic Data on Form I (Orthorhombic) of Streptovitamin A.* Crystal habit was tabular; optic sign, negative; optic axial angle,  $2V = 28^\circ$ ; refractive indices (5893 Å):  $\alpha = 1.500$ ,  $\beta = 1.590$ ,  $\gamma = 1.596$ ; dispersion slight,  $r > v$ .

*Crystallographic Data on Form II (Monoclinic) of Streptovitamin A.* Crystal habit was acicular; optic sign, negative; optic axial angle,  $2V = 16^\circ$ ; refractive indices (5893 Å):  $\alpha = 1.496$ ,  $\beta = 1.593$ ,  $\gamma = 1.596$ ; dispersion slight,  $r > v$ ; roentgen-ray data, interplanar spacings, Å (Cu,  $k\alpha_1$ ): 12.27, 7.45, 6.78, 6.37, 5.98, 5.60, 5.35, 4.98, 4.69, 4.41, 4.25, 4.00, 3.88, 3.75, 3.47, 3.31, 3.17, 3.02, 2.89, 2.80, 2.71, 2.58, 2.54, 2.44, 2.35, 2.28, 2.22, 2.18, 2.09, 2.03, 1.94, 1.88.

#### SUMMARY

The isolation and characterization of streptovitamin A and related materials has been described. The crystalline substances are unique in possessing no antibacterial activity, limited antifungal activity and, especially for streptovitamin A, great antitumor activity. Streptovitamins A and B appear to be isomers having the molecular formula of  $\text{C}_{15}\text{H}_{23}\text{NO}_5$ .

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#### BIBLIOGRAPHY

1. FIELD, J. B.; COSTA, F., AND BORYCZKA, A.: Origin of a new antitumor agent, streptovitamin. *In: Antibiotics Annual 1958–1959*, New York, Medical Encyclopedia, Inc., 1959, p. 547.
2. EVANS, J. S.; MENGEL, G. D.; CERU, J., AND JOHNSTON, R. L.: Biological studies on streptovitamin A, a new antitumor agent. *In: Antibiotics Annual 1958–1959*, New York, Medical Encyclopedia, Inc., 1959, p. 565.
3. FIELD, J. B.; MIRELES, A.; PACHL, H. R.; BASCOY, L.; CANO, L., AND BULLOCK, W. K.: Experimental evaluation of a new antitumor agent, streptovitamin A. *In: Antibiotics Annual 1958–1959*, New York, Medical Encyclopedia, Inc., 1959, p. 572.
4. SOKOLOSKI, W. T.; EILERS, N. J., AND SAVAGE, G. M.: Paper chromatography and microbiologic assay of the streptovitamins. *In: Antibiotics Annual 1958–1959*, New York, Medical Encyclopedia, Inc., 1959, p. 551.
5. HERR, R. R.: Structure studies on streptovitamins A and B. *In: Antibiotics Annual 1958–1959*, New York, Medical Encyclopedia, Inc., 1959, p. 560.
6. BOCK, R. M., AND LING, N. S.: Devices for gradient elution in chromatography. *Anal. Chem.*, 26:1543 (1954).

A new antitumor agent, streptovitamin A, and several companion products have been isolated from a *Streptomyces griseus* fermentation. Biological properties of these products and assay methods are reported in other papers in this series.<sup>1-4</sup> The isolation procedures and some characteristics of streptovitacins A and B have been described in the previous paper by T. E. Eble et al.<sup>5</sup> This paper describes the chemical and physical studies which establish the structures of these compounds as hydroxylated cycloheximide\* derivatives.

## DISCUSSION

In crude preparations encountered during the early isolation work, the streptovitacins appeared to be sugar-like, although a structural relationship to cycloheximide was also regarded as a possibility. Prior to obtaining pure crystalline streptovitamin A, initial degradation experiments were carried out on a crude preparation (approximately 40 per cent streptovitamin A) to investigate the possible relationship to cycloheximide.

Alkaline degradation of the crude material gave, in addition to ammonia, a steam-volatile product that would form carbonyl derivatives. The boiling range and infrared spectrum of this product suggested it to be a mixture of two ketones, one  $\alpha,\beta$ -unsaturated and the other a nonconjugated hydroxyketone. These were separated by chromatography on alumina and derivatives were prepared. The unsaturated ketone was identified from literature data as 2,4-dimethyl-2-cyclohexenone<sup>6</sup> (I) (fig. 1). Analyses of the semicarbazone and 2,4-dinitrophenylhydrazone derivatives of the hydroxyketone gave values consistent with a hydroxydimethylcyclohexanone structure (II). When crystalline streptovitamin A became available, a small sample was degraded with alkali, and the isolated product was shown by infrared analysis to be predominantly the hydroxyketone.

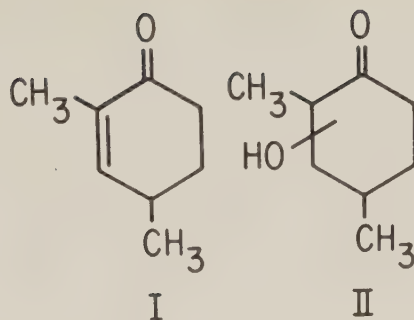
Dehydration of the hydroxyketone with strong acid gave 2,4-dimethyl-2-cyclohexenone. This suggested that the hydroxyl group in the original ketone is in the 2-, 3-, or possibly the 4-position. Since streptovitamin A is not oxidized by periodate, the hydroxyl group cannot be in the 2-position. The hydroxyketone gives a positive Fearon-Mitchell test for primary or secondary alcohols,<sup>7</sup> indicating the 3-position as the most likely. This was supported by spectrophotometric evidence that the compound is readily oxidized by chromic acid in 90 per cent acetic acid.

Elemental analysis of a recrystallized sample of streptovitamin A showed the empirical formula to be  $C_{15}H_{23}NO_5$ . This is equivalent to cycloheximide plus one oxygen. The rotatory dispersion curve of streptovitamin A in dioxane (fig. 2) is nearly identical to that of cycloheximide, again suggesting a close structural similarity. Although aqueous titration of streptovitamin A indicated no titratable groups, the imide group was readily detected by nuclear magnetic resonance spectra.

From this information, an obvious possible structure is the hydroxylated cycloheximide structure shown in fig. 3. It was reasoned that if this is correct, proper conditions of dehydration and rearrangement of double bonds should convert strep-

\* The trade name of The Upjohn Company for cycloheximide is Acti-dione.

FIG. 1. Ketone products from streptovitamin A are illustrated.



to vitamin A into a phenolic derivative previously prepared from cycloheximide. Such conditions were obtained by treating streptovitamin A with 50 per cent sulfuric acid. Infrared spectra showed the product to be identical to the cycloheximide derivative.

These properties appear to be most consistent with the structure 3-[2-(4-hydroxy-3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutarimide for streptovitamin A.

Elemental analyses of streptovitamin B showed the empirical formula of this product also to be  $C_{15}H_{23}NO_5$ . The rotatory dispersion curve (fig. 2) is similar in shape to those of cycloheximide and streptovitamin A, differing only in magnitude. Treatment of streptovitamin B with 50 per cent sulfuric acid gives the same phenolic derivative obtained from streptovitamin A. Alkali treatment of streptovitamin B afforded only the unsaturated ketone 2,4-dimethyl-2-cyclohexenone. Streptovitamin B, like streptovitamin A, is not oxidized by periodate. From these results, it is apparent that streptovitamin B is an isomer of streptovitamin A.

#### EXPERIMENTAL STUDIES

*Alkaline Degradation.* Thirty Gm. of a crude streptovitamin preparation was dissolved in 600 ml. of 20 per cent sodium hydroxide and the solution was distilled until about 300 ml. of distillate was collected. This was saturated with sodium chloride and extracted with ether. The ether extract was dried over sodium sulfate and the ether was removed under reduced pressure. The oily residue was distilled at reduced pressure (13 mm.) and distillate was collected from 75 to 125 C. The distillate weighed 2.45 Gm. The infrared spectrum of the distilled oil showed OH absorption and two carbonyls, one conjugated and one nonconjugated.

A 100 mg. sample of crystalline streptovitamin A was dissolved in 5 ml. of 20 per cent sodium hydroxide and distilled. About 2 ml. of distillate was collected, saturated with sodium chloride, and extracted with ether. Removal of the ether by careful evaporation left 35 mg. of residual oil. The infrared spectrum indicated this product to be predominantly the hydroxyketone.

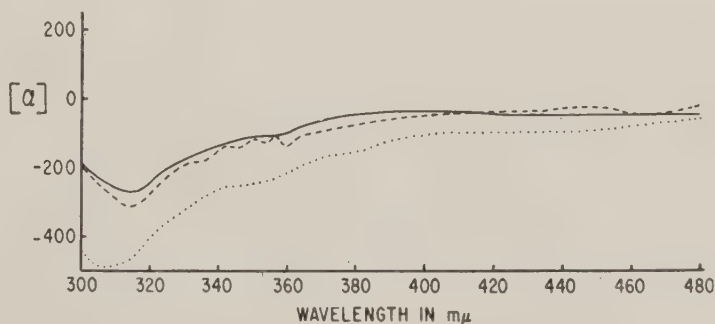


FIG. 2. Rotatory optical dispersion is shown.

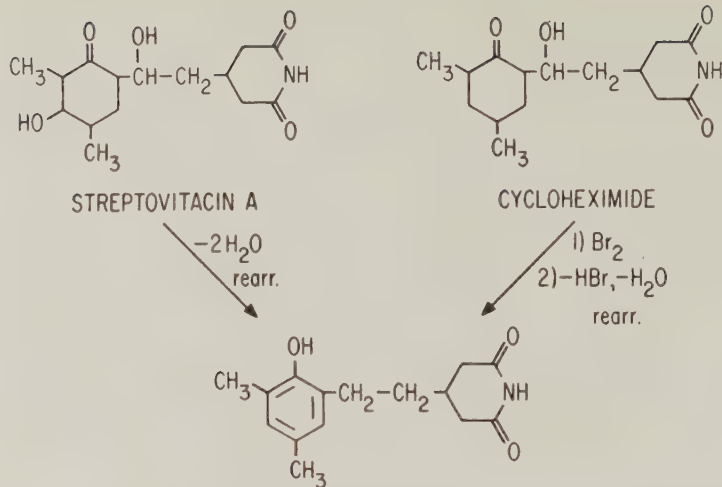


FIG. 3. The degradation of streptovitamin A is shown.

*Separation of Ketones.* The distilled mixed ketones (2.45 Gm.) described were chromatographed on a column containing 150 Gm. of alumina and packed in pentane-ether, 2:1. The column was eluted with the same solvent and 25 ml. fractions were collected. The residue from the first 10 fractions was shown by infrared spectra to be predominantly the conjugated ketone. The eluting solvent was changed to pure ether and 14 more fractions were collected. These contained a mixture of the conjugated and hydroxyketones. The solvent was now changed to 50 per cent methanol in ether and eight more fractions were collected. The residue from these fractions was shown by infrared spectra to be predominantly the hydroxyketone.

*Identification of the Unsaturated Ketone.* Collected data on the  $\alpha,\beta$ -unsaturated ketone showed this product to have a boiling point at atmospheric pressure of approximately 188 C., and an ultraviolet spectrum indicating a di-substituted double bond ( $\lambda_{\text{max}}$  236  $\text{m}\mu$ ,  $\epsilon = 10,500$ ). The ultraviolet spectrum of the 2,4-dinitrophenylhydrazone (m.p. 186 to 188 C.) confirmed the  $\alpha,\beta$ -unsaturated structure, and elemental analysis of the semicarbazone (m.p. 162—164 C.) indicated the empirical formula of the ketone to be  $\text{C}_8\text{H}_{12}\text{O}$ .

**ANALYTICAL.** Calculated for  $\text{C}_9\text{H}_{15}\text{N}_3\text{O}$ : C, 59.66; H, 8.34; N, 23.10; O, 8.83. Found: C, 59.89; H, 8.49; N, 22.73; O, 9.14.

Examination of the literature for compounds having the required structural features showed that 2,4-dimethyl-2-cyclohexenone gave a 2,4-dinitrophenylhydrazone which melted at 167—168 C.<sup>6</sup> Other isomeric dimethylcyclohexenones had quite different properties.

*Derivatives of the Hydroxyketone.* A 100 mg. sample of the hydroxyketone was dissolved in 1 ml. of 95 per cent ethanol and 100 mg. of 2,4-dinitrophenylhydrazine was added. The mixture was heated to boiling and five drops of concentrated hydrochloric acid was added. All solids went into solution and after heating for a few minutes the solution was chilled and scratched. A crystalline orange solid separated. After four recrystallizations from ethanol the sample weighed 27 mg. and melted at 139—141 C. The ultraviolet spectrum of this product showed a peak at 365  $\text{m}\mu$  indicating a derivative of a nonconjugated ketone.

**ANALYTICAL.** Calculated for  $\text{C}_{14}\text{H}_{18}\text{N}_4\text{O}_5$ : C, 52.17; H, 5.63; N, 17.39; O, 24.82. Found: C, 51.95; H, 5.46; N, 16.87; O, 24.59.

A 100 mg. sample of the hydroxyketone was dissolved in 1.5 ml. of water. To this was added 100 mg. of semicarbazide hydrochloride and 150 mg. of sodium acetate and the mixture was shaken until all solids had dissolved. The clear solution was

stoppered, put in a beaker of boiling water and allowed to cool overnight. No crystals formed, so the solution was chilled and scratched. Crystals formed after 30 minutes. After two recrystallizations from water, the semicarbazone weighed 33 mg. and melted at 177—179 C.

ANALYTICAL. Calculated for  $C_9H_{17}N_3O_2$ : C, 54.29; H, 8.61; N, 21.11; O, 16.08. Found: C, 54.35; H, 8.81; N, 20.25; O, 15.86.

*Dehydration of the Hydroxyketone.* A 67 mg. sample of the hydroxyketone was dissolved in 2 ml. of 50 per cent sulfuric acid and heated on the steam bath for five minutes. The solution became milky and slightly yellow. After cooling, the mixture was extracted three times with ether. Evaporation of the ether gave a light oil residue which was identified by its infrared spectrum as 2,4-dimethyl-2-cyclohexenone.

*Periodate Experiments with Streptovitamin A.* One ml. of a solution of 12 mg. of streptovitamin A in 50 ml. of water was diluted to 8 ml. The resulting solution was 0.1 mM. Five ml. of this solution was mixed with 5 ml. of a 0.4 mM solution of sodium periodate in pH 4.5 acetate buffer, quickly transferred to a spectrophotometer cell, and the absorption at 223  $m\mu$  was measured. Periodate absorbs at this wavelength. The absorption was constant over a 30 minute period, indicating no reaction was occurring.

In a qualitative test, lack of formation of the precipitate with silver nitrate showed that streptovitamin A caused no reduction of periodate to iodate after 12 hours in solution with periodic acid.

*Chromic Acid Oxidation of Hydroxyketone.*<sup>8</sup> A 2.3 mg. sample of the hydroxyketone was dissolved in 8.1 ml. of 90 per cent acetic acid. Five ml. of this 2 mM solution was mixed with 5 ml. of a 4 mM solution of chromic acid in 90 per cent acetic acid, the resulting solution was transferred to a spectrophotometer cell and the absorption at 360  $m\mu$  was measured. The absorption steadily decreased indicating reduction of the chromic acid. The reaction consumed more than 1 mole of chromic acid based on a control experiment with cyclohexanol. Cyclohexanone under these conditions showed essentially no reduction of chromic acid after 12 hours.

*Reaction of Streptovitamin A with 50 Per Cent Sulfuric Acid.* A 500 mg. sample of streptovitamin A was dissolved in 20 ml. of 50 per cent sulfuric acid and heated for five minutes on the steam bath. The solution was then poured into 100 ml. of water. After chilling in the refrigerator for an hour a crystalline precipitate separated. This was collected by filtration, washed with water, and air-dried to give 308 mg. of a white crystalline solid that melted at 135—145 C. A sample was recrystallized twice from water with a small amount of ethanol added. The recrystallized material melted at 148—150 C. A sample of the phenolic derivative prepared from cycloheximide by A. J. Lemin, and which melted at 148—155 C., was obtained. A mixture of these materials melted at 145—153 C. Infrared spectra showed the two products to be identical.

*Reaction of Streptovitamin B with 50 Per Cent Sulfuric Acid.* A 300 mg. sample of streptovitamin B was treated with 50 per cent sulfuric acid as described for streptovitamin A. A recrystallized sample of the white crystalline product melted at 147—150 C. Infrared spectra showed this material to be identical to the derivatives obtained from streptovitamin A and from cycloheximide.

*Alkaline Degradation of Streptovitamin B.* A 100 mg. sample of streptovitamin B was dissolved in 10 ml. of 10 per cent sodium hydroxide and let stand at room temperature for an hour. The mixture was extracted with ether. Evaporation of the dried ether extract gave 17 mg. of an oily residue. Infrared spectra showed this oil to be 2,4-dimethyl-2-cyclohexenone.

*Periodate Experiments with Streptovitamin B.* A 3.5 mg. sample of crystalline streptovitamin B was dissolved in 11.8 ml. of water and 1 ml. of the resultant solution was diluted to 10 ml. to give a 0.1 mM solution. Five milliliters of this solution was mixed with 5 ml. of a 0.4 mM solution of sodium periodate in pH 4.5 acetate buffer, quickly transferred to a spectrophotometer cell and the absorption at 223 m $\mu$  measured. The absorption was constant over a 60 minute period, indicating no periodate was being consumed.

In a qualitative test, streptovitamin B caused no reduction of periodate to iodate after 12 hours in solution with periodic acid.

#### SUMMARY

Chemical degradations are described and interpreted as most consistent with the structure 3-[2-(4-hydroxy-3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-glutaramide for streptovitamin A. From similar results it is shown that streptovitamin B is an isomer of streptovitamin A.

#### ACKNOWLEDGMENTS

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#### BIBLIOGRAPHY

1. FIELD, J. B.; COSTA, F., AND BORYCZKA, A.: Origin of a new antitumor agent, streptovitamin. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 547.
2. SOKOLSKI, W. T.; EILERS, N. J., AND SAVAGE, G. M.: Paper chromatography and microbiologic assay of the streptovitamins. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 551.
3. EVANS, J. S.; MENGEL, G. D.; CERU, J., AND JOHNSTON, R. L.: Biological studies on streptovitamin A, a new antitumor agent. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 565.
4. FIELD, J. B.; MIRELES, A.; PACHL, H. R.; BASCOY, L.; CANO, L., AND BULLOCK, W. K.: Experimental evaluation of a new antitumor agent, streptovitamin A. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 572.
5. EBLE, T. E.; BERGY, M. E.; LARGE, C. M.; HERR, R. R., AND JACKSON, W. G.: Isolation, purification, and properties of streptovitamins A and B. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 555.
6. BIRCH, A. J.: Reduction by dissolving metals. Part V, *J. Chem. Soc.* p. 1642, 1947.
7. FEARON, W. R., AND MITCHELL, D. M.: The nitro-chromic acid reaction for the detection of primary and secondary alcohols, with special reference to saccharides, *Analyst* 57:372, 1932.
8. SCHREIBER, J., AND ESCHENMOSER, A.: Über die relative geschwindigkeit der chromosäureoxydation sekundärer, alicyclischer alkohole. *Helv. chim. Acta*, 38:1529, 1955.

# Biological Studies on Streptovitacin A, a New Antitumor Agent

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In 1944, Cornman<sup>1,2</sup> showed that crude penicillin extracts damaged sarcoma cells in vitro at concentrations tolerated by normal cells. Since that time many workers have tested antibiotic beers for their antineoplastic activity, and an increasing number of compounds of microbiological origin, possessing antitumor activity, have been isolated and studied in one or more tumor systems.

It was observed by Field et al.,<sup>3</sup> working with sarcoma 180, and by the authors, working with Ehrlich ascites and Walker adenocarcinoma tumors, that a streptomycete fermentation produced an antitumor agent that could not be accounted for on the basis of its antibiotic content. We found that the activity was reproducible and was present in all the fermentations tested.

An assay procedure based on the inhibition of the Walker 256 tumor was developed and used to follow the fractionation studies until crystalline streptovitacin A was obtained.<sup>4-6</sup>

The activity of some of the intermediate fractions against a spectrum of tumors was determined. Fractions of intermediate purity show the same spectrum as the crystalline material. The results reported in this paper are with the crystalline material, unless otherwise stated.

The tumors used were: sarcoma 180, ascites and solid; Ehrlich carcinoma, ascites and solid; Walker adenocarcinoma, Murphy-Sturm lymphosarcoma, Guerin<sup>7</sup> and Jensen sarcoma. The ascites tumors were transferred by injecting intraperitoneally 0.25 ml. of fluid containing 4.5 million tumor cells. The mouse and rat solid tumors were transferred by injecting subcutaneously 0.1 to 0.2 ml. of a tumor cell suspension. Male and female Swiss mice weighing 18 to 22 Gm. each were used for these studies. Male Sprague-Dawley rats weighing 60 to 80 Gm. each were used for the rat tumors.

Intraperitoneal injection of the drug was started 16 to 20 hours ( $T_1$ ) after the tumors were transplanted, and treatment was continued once daily for seven days for mice and for 10 days for rat tumors. The effect of varied modes of administration and varied dosage schedules has been studied in a preliminary manner.

The effect of the drug on ascites tumors was evaluated by determining the fluid volume and packed tumor cell volume or by survival tests. The solid tumors in mice were either dissected out and weighed or measured in two diameters using calipers. The rat tumors were measured in two diameters. Appropriate controls with respect to age and sex of animals were run in each experiment.

The drug was administered in 1 per cent carboxymethylcellulose. Streptovitacin A was shown to be stable in aqueous solution for at least 30 days when kept at 4 C. All dosages are reported as mg./Kg. of body weight per day.

## RESULTS AND DISCUSSION

Streptovitacin A was studied in mice bearing the ascitic form of either sarcoma 180 or Ehrlich carcinoma, using the packed tumor cell volume technique. Treatment was started on either  $T_1$  or  $T_5$  and continued once daily for seven days. The tumor

TABLE I  
*Effect of Streptovitacin A on Ascitic Tumors*

Tumor	Preparation	Dose,* mg./Kg./ day	No. sacri- ficed	Original weight	Change in body weight	Fluid volume, ml.	Hemato- crit	Packed tumor cell volume
Ehrlich ascites	Streptovitacin A	0.025	9	19	+1	5.0	25.0	1.25
Ehrlich ascites	Streptovitacin A	0.05	10	20	+1	4.0	24.4	0.98
Ehrlich ascites	Streptovitacin A	0.1	10	21	0	2.0	15.7	0.31
Ehrlich ascites	Streptovitacin A	0.2	7	21	0	2.0	13.9	0.28
Ehrlich ascites	Tumor control	—	9	20	+1	6.0	41.0	2.46
Ehrlich ascites	Streptovitacin A	3.3	9	20	0	5.9	24.4	1.44
Ehrlich ascites	Streptovitacin A	10.0	10	21	+1	6.2	20.0	1.04
Ehrlich ascites	Streptovitacin A	33.0	9	21	0	4.7	17.6	0.76
Ehrlich ascites	Tumor control	—	30	21	+3	6.5	29.0	1.89
Sarcoma 180 ascites	Streptovitacin A	0.025	10	20	+2	6.0	17.2	1.03
Sarcoma 180 ascites	Streptovitacin A	0.05	10	20	+3	3.0	17.1	0.51
Sarcoma 180 ascites	Streptovitacin A	0.1	9	20	+2	3.0	17.1	0.53
Sarcoma 180 ascites	Streptovitacin A	0.2	10	20	+2	2.0	12.3	0.25
Sarcoma 180 ascites	Streptovitacin A	0.4	10	20	+1	0	14.1	tr.
Sarcoma 180 ascites	Tumor control	—	10	20	+3	7.0	29.5	2.07

\* Injected intraperitoneally on T<sub>1</sub> through T<sub>7</sub>. Sacrificed on T<sub>8</sub>.

cell volume was measured 24 hours after the last injection. Thirty  $\mu\text{g./Kg.}$  daily produced a 50 per cent decrease in the packed tumor cell volume when treatment was started on T<sub>1</sub> (table I). Many of the animals receiving the higher dosages of the drug have no fluid or evidence of tumor cells in their peritoneal cavity. The toxic effects of the drug are shown by the marked change in body weight as seen when the original weight is compared to the final weight after removal of the ascitic fluid.

Sarcoma 180 cells appear to be more sensitive to the drug than the carcinoma cells when mice with established ascitic tumors are treated with streptovitacin A (tables II and III). The volume of sarcoma 180 cells present after treatment with 0.2 mg./Kg. was lower than the volume present at the start of treatment. The cells present after treatment with 0.2 mg./Kg. were shown to be viable. Therefore, under these conditions the drug appears to be a carcinostatic rather than a carcinolytic agent at this dosage level.

We have noted that, in spite of the reduction in volume of tumor cells produced, an increase in volume of peritoneal fluid occasionally occurs when established ascites tumors are treated with streptovitacin A. This may indicate a blockage of the lymph

TABLE II  
*Effect of Streptovitacin A on Established Ehrlich Ascites Tumors*

Preparation	T <sub>0</sub> →T <sub>11</sub> dose	Day of sacrifice	Survivors	Original weight	Final weight	Fluid volume, ml.	Hematocrit	Packed tumor cell volume
—	—	5	10	21	30	3.0	30.0	0.90
		8	10	20	34	9.0	28.3	2.54
		12	8	20	40	20.0	10.6	2.12
		5	10	18	26	3.0	27.7	0.83
		12	9	19	35	18.3	11.9	2.18
Streptovitacin A	0.05	12	10	19	31	12.7	17.3	2.20
Streptovitacin A	0.10	12	9	19	30	11.3	17.4	1.97
Streptovitacin A	0.20	12	8	20	28	8.8	19.9	1.75

TABLE III  
*Effect of Streptovitamin A on Established Sarcoma 180 Ascites Tumors*

Preparation	T <sub>0</sub> →T <sub>11</sub> dose	Day of sacrifice	Survivors	Original weight	Final weight	Fluid volume, ml.	Hematocrit	Packed tumor cell volume
Streptovitamin A	0.05	5	10	21	26	2.0	34.4	0.69
		8	10	21	34	7.0	31.0	2.17
		12	7	21	35	14.0	17.6	2.47
		5	10	19	24	1.8	32.7	0.59
		12	6	19	30	11.3	18.5	2.09
		12	8	20	30	10.6	18.3	1.94
		12	10	20	30	11.0	17.1	1.89
		12	10	18	27	9.1	8.1	0.74
		12	10	18	27	9.1	8.1	0.74
		12	10	18	27	9.1	8.1	0.74

system or that a toxic substance may have been liberated by the tumor, which produces capillary damage that is not reversible.

Survival tests were conducted by injecting streptovitamin A intraperitoneally on T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub> into groups of 20 ascitic female mice. The drug had no effect on the survival of mice with either sarcoma 180 or Ehrlich ascites tumors. When streptovitamin A was added to the drinking water in concentrations of 5 μg./ml. or more, there was an immediate decrease in the volume of water consumed by both normal and tumor-bearing mice. Mice receiving 5 μg./ml. in their drinking water for nine days survived 46 per cent longer than the controls. The effect of restricting water intake on the survival of ascitic mice was not controlled in this experiment, and the increase in survival time may be associated with the fluid intake.

The effect of streptovitamin A on the solid form of Ehrlich carcinoma was studied using groups of 20 animals. Therapy was begun on T<sub>1</sub> and continued daily for seven days. The results shown in table IV indicate that the drug is capable of producing a 70 per cent inhibition of the tumor but only at levels that cause a marked loss in body weight.

Four-tenths to 0.8 mg. of streptovitamin A produced an inhibition of sarcoma 180 when treatment was started on T<sub>1</sub>. The most effective levels were near the toxic range

TABLE IV  
*Effect of Streptovitamin A on Ehrlich Carcinoma (Solid)*

	Dose	Change in body weight,	Survivors	Average tumor weight, mg.	% inhibition
		Gm.			
Carboxymethylcellulose control	—	+2.7	20	301	—
Streptovitamin A	0.2	+2.1	20	343	0
	0.4	+0.6	20	233	23
	0.8	—5.2	17	91	70
	0.8	—5.2	17	91	70

TABLE V  
*Effect of Streptovitamin A on Sarcoma 180*

Preparation	Dose	Therapy	No. mice	No. survivors	Original weight	Final weight	Tumor weight	% inhibition
Streptovitamin A	0.8	—	15	15	21	23	781	—
		T <sub>1</sub> →T <sub>7</sub>	15	12	21	17	98	87
		—	30	30	21	24	775	—
Streptovitamin A	0.2	T <sub>1</sub> →T <sub>7</sub>	10	10	20	23	639	18
Streptovitamin A	0.4	T <sub>1</sub> →T <sub>7</sub>	10	8	20	22	282	64
Streptovitamin A	0.8	T <sub>1</sub> →T <sub>7</sub>	10	1	22	—	—	Toxic

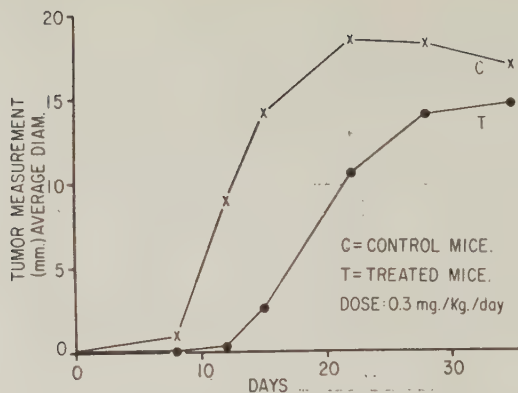


FIG. 1. The effect of streptovitacin A on sarcoma 180 is illustrated. The control group consisted of 92 mice; the treated, 90. Therapy was 0.3 mg./Kg./day,  $T_1$  through  $T_{21}$ .

(table V). Streptovitacin A was given at 0.3 mg./Kg. daily to a group of 90 tumor-bearing mice, beginning on  $T_1$  and continuing through  $T_{21}$ . The dosage, 0.3 mg./Kg./day, permitted the treated animals to maintain their own weight. At this dosage level, streptovitacin A caused a marked delay in the appearance of measurable tumors, and the average tumor size was smaller throughout the treatment and observation periods (figs. 1, 2, 3). The survival patterns of the treated and control groups were similar, with the treated group being slightly longer.

The Walker adenocarcinoma was used as the test system for following the isolation of streptovitacin A. The dose response of this tumor is linear when the level of the drug is kept below toxic levels. Paired feeding control experiments have shown that the antitumor activity of streptovitacin A cannot be accounted for by the inhibition of food intake. The degree of inhibition produced by a given dosage of streptovitacin A varies with each experiment (table VI).

Attempts to treat established Walker tumors were made. Initial studies on the crystalline material were made using 0.6 mg./Kg. Therapy was on an individual rat basis and was suspended when toxicity was evident. It was found that at this dosage level, frequent deaths occurred, some within a few hours, others after a two to six day time interval. Three animals that had received several doses of streptovitacin A were sacrificed and the following observations noted: Blood pigments were found in the bladder urine; the intestines were free of any solid material; the body was dehydrated and free of body fat; animals appeared grossly anemic with possibly some dysfunction of the blood-clotting mechanism.

Subsequent studies showed that a single dose of 0.6 mg./Kg. produced 16 hours later a significant prolongation of the clotting time. Six-tenths mg./Kg. rapidly re-

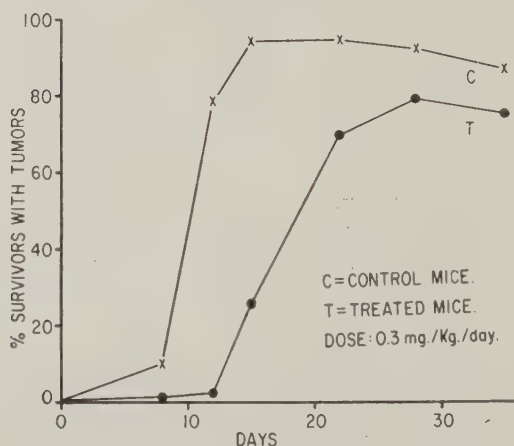
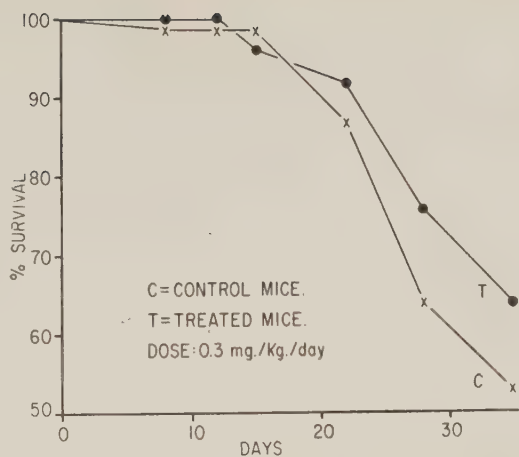


FIG. 2. The effect of streptovitacin A on sarcoma 180 is further illustrated. The control group consisted of 92 mice; the treated, 90. Therapy was 0.3 mg./Kg./day,  $T_1$  through  $T_{21}$ .

FIG. 3. The per cent of survival after treatment of sarcoma 180 with streptovitamin A is shown. The control group consisted of 92 mice; the treated, 90. Therapy was 0.3 mg./Kg./day, T<sub>1</sub> through T<sub>21</sub>.



duced the blood pressure in rats made hypertensive to desoxycorticosterone acetate. Rats that were hypertensive to desoxycorticosterone acetate responded to dosages as low as 0.2 mg./Kg. The drug could be given either orally or intraperitoneally. The drug produced a prolonged gradual drop in blood pressure in the normal dog. Congestion of the blood vessels in the mesentery was the most noticeable immediate effect.

Streptovitamin A was administered at 0.2 mg./Kg. daily in carboxymethylcellulose to groups of rats beginning with T<sub>1</sub> and as soon as the tumors had reached 5 mm. in average diameter. A few of the animals showed regressions of their tumors. This effect was more than balanced by the fact that 60 per cent of the animals died. Many of these deaths occurred while the animals were showing progressive weight gains and little or no evidence of toxic symptoms, such as diarrhea or anorexia.

Streptovitamin A was administered to rats bearing the Murphy-Sturm lympho-sarcoma with no evidence of effectiveness.

Streptovitamin A was administered to rats bearing the Guerin adenocarcinoma, which metastasizes routinely to the lymph nodes. The primary tumor was inhibited and the appearance of the metastatic lesions was delayed.

Streptovitamin A was administered in daily dosages of 0.1 to 0.2 mg./Kg. to rats with the Jensen sarcoma starting on T<sub>1</sub>. In one experiment in which the drug was given for 17 days (table VII), the tumors grew at a slow rate for 28 days and then began to regress. On the fiftieth day, 90 per cent of the animals were still surviving and only 1 animal out of 9 survivors showed a tumor. The 8 animals whose tumors had regressed would not accept a second tumor implant. A repeat on this experiment shows a similar regression of the tumor taking place when the drug was administered either intraperitoneally or orally. Streptovitamin A produced regressions when

TABLE VI  
Effect of Streptovitamin A on Walker 256  
Vehicle: Carboxymethylcellulose, Sprague-Dawley Male Rats

Dose, mg./Kg./day	% inhibition of tumor*		% inhibition of body weight gain*	
0.05	23, 42	(33)	10, 13	(12)
0.10	37, 38, 44, 59	(45)	29, 6, 31, 43	(27)
0.20	64, 74, 91, 72	(75)	54, 20, 57, 53	(46)
0.40†	67, 77	(66)	72, 41	(57)

\* Figures in parentheses are average values.  
† Dose could not be given daily because of toxicity.

TABLE VII  
Effect of Streptovitacin A on Jensen Sarcoma

Dosage schedule*	T <sub>6</sub>					T <sub>29</sub>				T <sub>57</sub>			
	T <sub>0</sub> body wt.	No. sur- vivors	No. tumors	Av. body wt.	Av. tumor measure- ment	No. sur- vivors	No. tumors	Av. body wt.	Av. tumor measure- ment	No. sur- vivors	No. tumors	Av. body wt.	Av. tumor measure- ment
Control carboxy- methylcellulose 5 ml./Kg. T <sub>1</sub> through T <sub>17</sub>	84	9	8	184	31.5	8	7	243	42.2	3	1	345	8.0
Streptovitacin A 0.05 mg./Kg. T <sub>1</sub> →T <sub>17</sub>	83	10	9	159	31.3	9	8	198	33.5	4	0	321	0.0
0.1 mg./Kg. T <sub>1</sub> →T <sub>17</sub>	84	10	10	147	29.0	9	9	182	34.6	4	0	322	0.0
0.2 mg./Kg. T <sub>1</sub> →T <sub>17</sub> except T <sub>1</sub> , T <sub>6</sub>	82	9	6	130	14.3	9	6	177	13.3	9	1	309	1.9
0.4 mg./Kg. T <sub>1</sub> →T <sub>17</sub> except T <sub>4</sub> , T <sub>6</sub> , T <sub>10</sub> , T <sub>17</sub>	83	9	7	109	17.5	5	2	163	7.5	5	0	303	0.0

\* Ten animals were used for each level.

treatment was started immediately (T<sub>1</sub>) or when the tumor was approximately 5 mm. in average diameter.

Concurrent administration of high doses of vitamins, serving as cofactors in many enzyme systems, did not reduce the toxic effects of streptovitacin A or reduce the antitumor activity. The regular administration of the water-soluble analogue of vitamin K, tetrasodium salt of 2-methyl-1,4-naphthoquinone diphosphoric acid, had no effect on the antitumor activity but may have improved slightly the gross appearance of the animals.

### SUMMARY

Streptovitacin A has been tested against two mouse and four rat tumors. Five out of six of the tumors were inhibited when treatment was started within 24 hours after the tumors were planted.

Additional studies are needed on dosage schedules to permit prolonged treatment of established tumors.

The toxic symptoms noted at the high dosage levels were listlessness, diarrhea, and hematuria.

### ACKNOWLEDGMENT

Grateful acknowledgment is hereby given to Drs. N. Guerin and Charles Oberling, of the Institut de Recherches sur le Cancer, de l'Université de Paris, Paris, France, for the Guerin tumor; to Dr. W. R. Graham for pathology studies; and to Drs. W. Freyburger and J. Correll for blood pressure studies. Streptovitacin A was supplied by Drs. T. Eble and R. Herr, of the Department of Microbiology, The Upjohn Co.

### BIBLIOGRAPHY

- CORNMAN, I.: Survival of normal cells in penicillin solutions lethal to malignant cells, *Science* 99:247, 1944.

2. CORNMAN, I.: A selective lethal effect of penicillin on sarcoma 180 cells growing with normal tissue in roller-tube cultures, *J. Gen. Physiol.* 28:113, 1944.
3. FIELD, J. B.; COSTA, F., AND BORYCZKA, A.: Origin of a new antitumor agent, streptovitacin. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 547.
4. EBLE, T. E.; BERGY, M. E.; LARGE, C. M.; HERR, R. R., AND JACKSON, W. G.: Isolation, purification, and properties of streptovitacins A and B. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 555.
5. SOKOLSKI, W. T.; EILERS, N. J., AND SAVAGE, G. M.: Paper chromatography and microbiological assay of the streptovitacins. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 551.
6. HERR, R. R.: Structure studies on streptovitacins A and B. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 560.
7. GUERIN, M., AND GUERIN, P.: Epithelioma de l'uterus du rat, lymphotrope et transplantable, *Bull. French Assoc. Cancer Research* 23:1, 1934.

# Experimental Evaluation of a New Antitumor Agent, Streptovitacin A

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In the preceding reports are detailed the series of events leading to streptovitacin A, from the discovery in a bioassay of antitumor activity of a crude *Streptomyces griseus* fermentation to the isolation of a crystalline material. This paper concerns itself with a preliminary report of some observations on the biological properties of streptovitacin A in normal and tumor-bearing mice and rats.

## EXPERIMENTAL

**Methods.** COMPOUND. The streptovitacin A was dissolved in sterile physiological saline solution. It was given in a volume of 0.5 ml. to mice and 1 ml. to rats. Various dose regimens and routes were utilized as stated.

**TUMOR TYPES AND THEIR PREPARATION.** A variety of tumors were used in these studies. In the mouse they included the Crocker sarcoma 180, RC carcinoma, leukemia L 4946, and the Ehrlich ascites tumors. In the rat, the Walker 256 carcino-sarcoma was studied. In the cases of the parenchymal tumors, they were 7 day old growths and were divided into equal fragments of viable and non-necrotic tissue under aseptic conditions. The portions measured about 1.5 mm. in all dimensions and were implanted by trocar subcutaneously into the axilla of healthy mice.

The Walker carcino-sarcoma of rats was managed by combining several 14 day non-necrotic tumor growths, mincing the tumor in a homogenizer under aseptic conditions and injecting 0.4 ml. of a 1:5 cell suspension into the axilla of the rat.

The Ehrlich ascites tumor was obtained by pooling the ascitic fluid from 3 or more mice in the seventh day of tumor growth and injecting 0.1 ml. of the undiluted cell-bearing fluid into each mouse under aseptic conditions. Cell counts indicated that the inoculum contained about 500,000 to 800,000 tumor cells.

**ANIMALS USED.** The mice were 18 to 22 Gm. in weight and were maintained on an ad libitum diet of Purina laboratory chow, barley, and water. Female white Swiss mice were used both for the sarcoma 180 and Ehrlich ascites tumors, female AKR mice were used for leukemia L 4946 and female DBA mice were used for the RC carcinoma. Female Wistar rats 80 to 100 Gm. in weight were used for the Walker carcino-sarcoma.

**MEASUREMENT OF EFFECT UPON TUMORS.** All animals were weighed once weekly and prior to taking measurements of tumors. The parenchymal tumors (sarcoma 180, RC carcinoma, L 4946 leukemia, and Walker carcino-sarcoma) were evaluated primarily by measurement of size. This was done during the course or at the conclusion of the study period, by measuring with calipers the two diame-

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TABLE I  
Effect of Streptovitacin A on Sarcoma 180

Treatment	Daily dose, mg./Kg./day	Route	No. mice	No. treat.	Average wt. change,* Gm.	Degree of tumor inhibition: average reduction from control in %	
						Size	Weight
Streptovitacin A	0.01	Intraperitoneal	10	7	—2.5	54	—
	0.2	Intraperitoneal	10	6	—1.5	50	91
	0.5	Intramuscular	10	6	—4.1	66	92
Amethopterin	0.75	Intraperitoneal	10	5	—3.3	45	88
Streptovitacin A	0.1	Intraperitoneal	20	7	—1.7	25	
	0.1	Intraperitoneal	10	7	+3.5	47	66
	0.2	Intraperitoneal	20	7	—2.9	38	
	0.5	Intraperitoneal	20	7	—4.4	57	
	0.5	Intraperitoneal	10	5	—3.0	61	83
Azaserine	20.0	Intraperitoneal	10	7	—3.8	54	
Streptovitacin A	0.6	Subcutaneous	10	6	—1.9	48	77
	1.2	Subcutaneous	10	4	—4.0	66	80
	1.2	Oral	10	5	—5.8	74	86
Triethylene melamine	4.0	Intraperitoneal	10	6	—4.5	65	79

\* Saline injected control mice have changes in weight of from +1.0 to —2.0 Gm. after eight days.

ters of the tumors, calculating the average tumor dimensions of all animals in the experimental group and then comparing the treated and control groups. In many instances, and where possible, this technique was supplemented by sacrificing the animal, excising the whole tumor, cleaning, and weighing it. Customarily unless otherwise indicated, the mouse tumors were evaluated on the eighth day of growth, and the Walker tumor of the rat on the eighth and fifteenth days. A level of significance is considered established when the tumors of the test animals measure at least 25 per cent less than the controls. The greater the inhibition the smaller the tumor as compared with the control.

The antitumor effect in mice with Ehrlich ascites was evaluated by recording the survival time. An average was obtained of the survival time of all mice in a group and this was compared to saline injected control mice. A prolongation of life beyond 20 or 25 per cent is considered significant. The mice with leukemia L 4946 were also evaluated not only by survival times, but also white blood and differential cell counts were taken routinely at 2 or 3 day intervals until the mice succumbed.

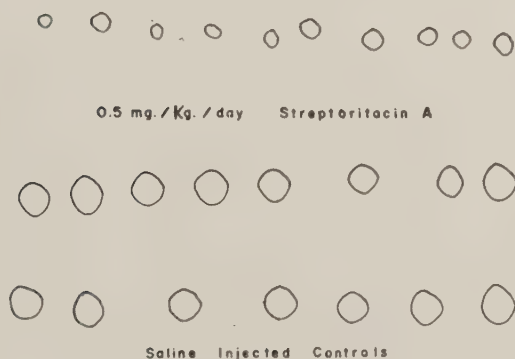


FIG. 1. Outline of size of eight day sarcoma 180 tumors is shown. The tumors from mice treated with streptovitacin A are compared with those from control mice.

TABLE II  
*Effect of Streptovitacin A on RC Carcinoma*

Treatment	Daily dose, mg.	Route	No. mice	No. treat.	Average wt. change,* Gm.	Degree of tumor inhibition: average reduc- tion from control in %	
						Size	Weight
Streptovitacin A	0.6	Intramuscular	10	7	—1.9	41	61
	1.2	Intramuscular	10	7	—2.3	40	63
Triethylene melamine	0.75	Intraperitoneal	10	5	—4.3	70	—
Streptovitacin A	0.2	Intraperitoneal	15	7	—0.5	28	36
	0.5	Intraperitoneal	15	7	—0.8	35	48
Triethylene melamine	0.75	Intraperitoneal	10	6	—1.5	54	—

\* Saline injected control mice have changes in weight of from +1.0 to —2.0 Gm. after eight days.

In the addition to saline injected control mice this laboratory routinely tests new agents by comparing their effects with antineoplastic agents of known potency. This has been done in the present investigation with the use of amethopterin, triethylene melamine, and azaserine as shown.

**PATHOLOGICAL STUDIES.** Careful autopsy examination was made of a significant sampling of mice or rats from all groups studied. Specimens were removed from vital organs. A careful histological examination was made of all organs and tumors.

## RESULTS

*Effect of Streptovitacin A on Sarcoma 180.* EFFECT OF DRUG GIVEN ONCE DAILY. The results of several tests where the agents were administered once daily are summarized in table I. Several different routes of administration are included and for comparison, results of tests with amethopterin, azaserine, and triethylene melamine are also included. It would appear that by any route utilized, intramuscular, subcutaneous, intraperitoneal, or oral, a very significant inhibition of the sarcoma 180 resulted. Whereas 4 mg./Kg./day of triethylene melamine reduced sar-

TABLE III  
*Effect of Streptovitacin A on Ehrlich Ascites Tumor*

Treatment	Daily dose, mg./Kg./day	Route	No. mice	No. treat.	Average survival	Prolongation of survival
					Days	Average %
Streptovitacin A	0.6	Intraperitoneal	14	7	13.9	33
	0.6	Intramuscular	14	7	12.6	29
	1.2	Subcutaneous	14	7	12.5	28
Saline solution	—	Intraperitoneal	14	7	9.8	—
Streptovitacin A	0.01	Intraperitoneal	10	7	16.1	24
	0.02	Intraperitoneal	10	7	15.4	18
	0.1	Intraperitoneal	10	7	16.7	37
Amethopterin	0.75	Intraperitoneal	10	7	19.3	48
Saline solution	—	Intraperitoneal	10	7	13.0	—

TABLE IV  
*Effect of Streptovitamin A on Walker 256 Carcino-Sarcoma\**

Treatment	Daily dose, mg./Kg./day	No. rats	No. deaths	No. treat.	Degree of tumor inhibition average reduction from control in %
8 Day Tumors					
Streptovitamin A	0.75	12	2	6	63
Amethopterin	0.5	12	6	7	48
15 Day Tumors					
Streptovitamin A	0.2	12	0	7	50
	0.75	12	6	6	36

\* Drugs given intraperitoneally.

coma 180 tumors to 65 per cent of the size and 79 per cent of the weight of controls, essentially the same result was obtained with 0.5 mg. of streptovitamin A, with which the tumors measured 66 per cent less in size and 92 per cent less in weight of the control tumors. In figure 1 appears a graphic representation of the size of the drug-treated tumors as compared to the controls.

EFFECT OF VARIATIONS IN REGIMEN OF DRUG ADMINISTRATION. When the agent was given at levels of 0.1, 0.2, or 0.5 mg./Kg./day intraperitoneally, the degree of tumor inhibition was the same whether this was accomplished in one single injection or two injections a day.

When larger doses of 1 or 2.5 mg./Kg. were given in a single dose one day after tumor implantation, the eight day tumor showed no measurable inhibition. When the mice were given 1 mg./Kg./day every other day, the eight day tumor was only slightly inhibited.

EFFECT OF STREPTOVITACIN A ON ESTABLISHED TUMORS. Excellent inhibition was easily demonstrable in mice when drug injections began one day after tumor implantation. However, when mice with tumors that had been allowed to grow for two, three, or four days were given daily intraperitoneal injections of 0.2 or 0.5 mg./Kg./day of streptovitamin A, a lag in growth of the tumor was observed for about two days in each case, and then the tumor growth increased in a variable fashion during the remainder of the seven day injection period. Nevertheless, the tumors of mice whose treatment with streptovitamin A began when the tumor was two, three, or four days established had a final tumor size on the tenth day of tumor growth about 20 per cent less than that of the controls.

TABLE V  
*Effect of Streptovitamin A on Leukemia L 4946\**

Treatment	Daily dose, mg./Kg./day	No. mice	No. treat.	Degree of tumor inhibition	
				Average reduction from control size	Average prolongation of life over control
Streptovitamin A	0.1	15	14†	35	19
Amethopterin	0.75	15	14	79	107
Streptovitamin A	0.3	15	5	32	—
	0.4	15	4	49	0
	0.5	45	5	44	5
	0.6	15	4	42	—

\* All treatments were given intraperitoneally.

† Given twice daily.

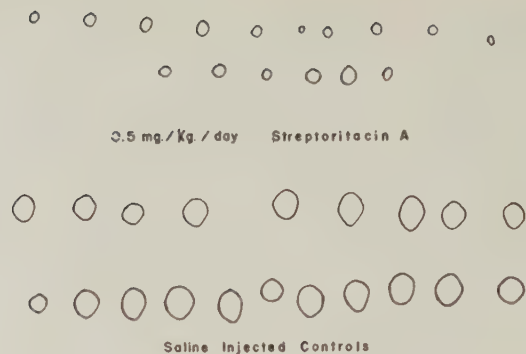


FIG. 2. Outline of size of eight day leukemia L 4946 tumors is given. The tumors from mice treated with streptovitamin A are compared with those from control mice.

EFFECT OF INTRATUMOR INJECTIONS OF STREPTOVITACIN A. Mice with eight day sarcomas received daily injections of 0.1 ml. containing 0.25 or 1.0 mg./Kg./day of streptovitamin A directly into the substance of the tumors. This was accomplished with a 27 gauge needle. Control mice received intratumor injections of saline solution. Tumor measurements were made in the usual way every second day for six days. No significant tumor inhibition was observed.

EFFECT OF CONTACT OF TUMOR WITH STREPTOVITACIN A ON ITS VIABILITY. Uniform portions of 1.5 mm. diameter from one tumor were prepared aseptically in Petri dishes in the usual manner. They were then bathed in Ringer's solution containing 0.1 mg./ml. of streptovitamin A for one and three hours while under partial refrigeration. Suitable control tumor portions were bathed in Ringer's solution and all were implanted in mice in the manner previously stated. Tumor measurements made on the eighth day of growth showed no significant difference amongst the groups.

ABILITY OF STREPTOVITACIN A INHIBITED TUMOR TO REGENERATE. Portions of tumor were prepared and implanted in the usual way from the eight day tumors of mice given 0.1 and 0.5 mg./Kg./day of streptovitamin A or saline solution. The degree of tumor inhibition in size had been 47 and 61 per cent respectively for the drug-treated tumors. After eight days the new growths were measured. The implant from the 0.1 mg./Kg./day drug-treated mice had grown to the size of the controls while the 0.5 mg./Kg./day treated implants were 40 per cent smaller in size.

EFFECT OF STREPTOVITACIN A ON SURVIVAL OF TUMOR-BEARING MICE. Separate groups of mice with sarcoma 180 were treated with 0.01, 0.02, 0.5, and 1.0 mg./Kg./day of streptovitamin A for seven daily injections. Their total survival was compared with saline-injected control tumor-bearing mice. Although levels of 0.1 mg. and greater of the drug produced a considerable tumor inhibition, the survival time of the mice was not significantly greater than that of the control mice.

*Effect of Streptovitamin A on RC Carcinoma.* The RC carcinoma was inhibited

TABLE VI  
White Blood Cell Counts in Leukemia L 4946

Treatment	Dose, mg./Kg./day	Day of leukemia, average count per cu. mm.		
		4	7	9
Streptovitamin A	0.1	18,000	48,600	67,600
	0.4	5,900	35,300	52,800
Control	—	13,300	17,300	67,200

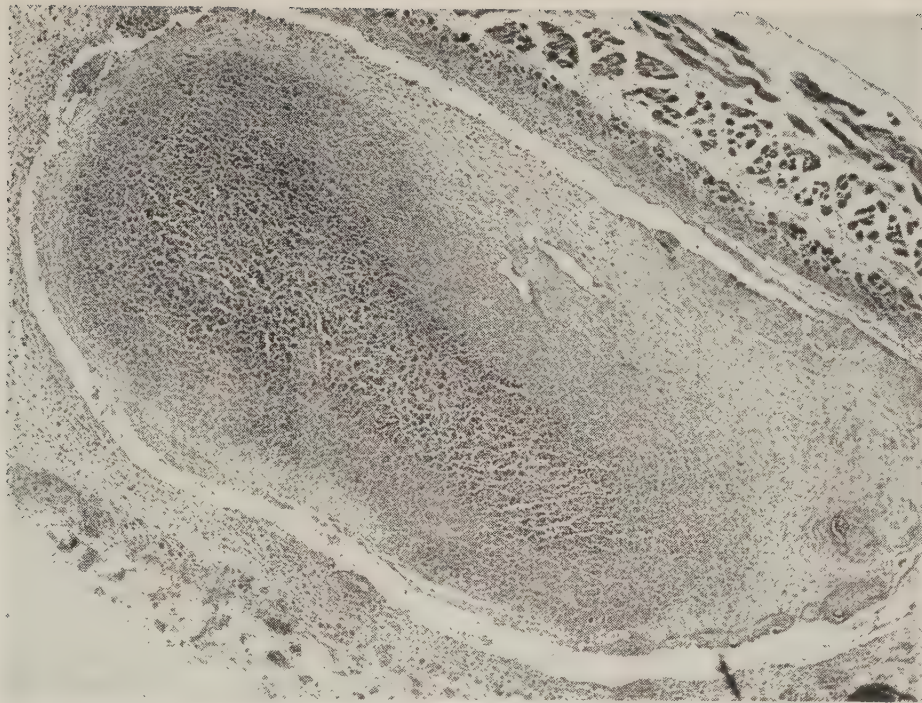


FIG. 3. Shown is cross section of sarcoma 180 from mouse treated with 0.2 mg./Kg./day for seven days, low power. Extensive necrotic changes are evident with viable tumor present through the center.

but not to the same degree as the sarcoma 180, when the mice were given streptovitamin A. Table II summarizes some of the results with this tumor. A level of 0.6 mg./Kg./day given intramuscularly for seven days inhibited the tumor to 41 per cent of the size of its control.

*Effect of Streptovitamin A on Ehrlich Ascites Tumor.* The drug was given by all routes of administration and for seven days to mice with Ehrlich ascites tumor and the survival period was recorded. A significant prolongation of life was obtained and the results are summarized in table III.

*Effect of Streptovitamin A on Walker 256 Carcino-Sarcoma.* The new agent was administered by intraperitoneal injection for seven days beginning one day after tumor implantation to rats with the Walker carcino-sarcoma. Tumor sizes were measured at the end of the injection period and again one week later. The data are given in table IV. It will be seen that streptovitamin A compared well with amethopterin.

*Effect of Streptovitamin A on Leukemia L 4946.* Several techniques are available for the management of this tumor. It was decided to combine its study both as a solid tumor and also record the survival time, since in time the mouse dies of systemic leukemia. White blood studies were also made. In table V is summarized the results of some of these studies and a comparative result with amethopterin is included. More graphically the results are also shown in figure 2 where tumor sizes are compared. It is apparent that in this leukemia, streptovitamin A significantly inhibits the growth of the solid tumor but does not prolong the life of the leukemia mice. Its total effect was considerably less impressive than that of amethopterin.

In table VI is given a summary of some of the total white blood counts of the leukemic mice. It was of interest to note the early leukopenia in the mice given

0.4 mg./Kg./day, since a temporary leukopenia has been observed initially in normal mice receiving a large single dose of streptovitacin A, and a leukocytosis has appeared in normal mice receiving smaller doses over a period of one week or more. The drug otherwise did not significantly affect the count of mice with leukemia L 4946.

**HISTOPATHOLOGICAL STUDIES.** The administration of relatively large doses of streptovitacin A to mice produced no marked or consistent change in any of the vital organs studied to date. Occasionally a mild toxic myocarditis has been seen. When given to mice with the tumors described alterations observed were consistent with gross liquefactive changes seen at autopsy. Generally when a tumor was significantly inhibited in growth it was found to have an increase of areas of necrosis with reduction in mitotic figures, and the presence of pyknosis of nuclei, karyorrhexis, and karyolysis. This was similar in degree to the changes induced in the same tumors by other agents of known antitumor effectiveness. An example of a cross section of a sarcoma 180 from mouse treated with streptovitacin A is given in figure 3. The marked generalized necrosis of the tumor is evident.

#### DISCUSSION

The preliminary evaluation of some of the biological properties of streptovitacin A indicates that it may have a wide spectrum of antitumor effectiveness. The present report gives five different tumor types in which some degree of significant effect has been achieved. Other trials still under way here will show that this spectrum can be broadened. In this laboratory as yet, no tumor type in which the new agent was tested, has been unaffected. As expected, however, the degree of tumor inhibition has been variable. The best results have been achieved here with sarcoma 180.

The new agent possessed activity regardless of route of administration. It was effective orally as well as parenterally. Although the greatest degree of tumor inhibition was a dose approximating 0.5 mg./Kg./day, a level that exhibited some degree of intoxication, good tumor inhibition was frequently observed at a dose of 0.01 mg./Kg./day. At the latter level, excepting for slight loss in weight, the general health of the animals did not seem to be impaired as tested by other criteria.

In general streptovitacin A appears to be comparable with other antitumor agents, such as amethopterin, triethylene melamine, and azaserine. This is particularly true in light of the results of the studies with sarcoma 180. Based on the small dose required, streptovitacin A might be considered to be a potent tumor inhibitor.

It would appear from these preliminary results that streptovitacin A is a growth inhibiting agent, but not carcinolytic. Thus not only are well-established animal tumors not so significantly affected as are the freshly transplanted tumors, but also tumors inhibited by the new agent will resume a considerable measure of growth.

In addition to other tumor systems being evaluated, trials are now under way in dogs with spontaneous tumors. It is anticipated that clinical evaluation in human beings may be initiated soon.

#### SUMMARY

1. Streptovitacin A has been evaluated in a preliminary fashion in a group of transplantable mouse and rat tumors. These include the sarcoma 180, RC carcinoma, leukemia L 4946, Ehrlich ascites, and Walker 256 carcino-sarcoma. A significant inhibition was induced in all tumors studied.

2. In sarcoma 180 the degree of tumor inhibition was comparable to that of a

number of known antitumor agents. The daily administration of the new agent in doses of 0.5 mg./Kg./day resulted in tumors measuring about 60 per cent less than controls. At a level of 0.01 mg./Kg./day significant tumor inhibition was observed.

3. Streptovitamin A possessed antitumor effectiveness whether given orally or by several parenteral routes. It had no effect on injection directly into tumors. Although inhibited tumors from mice treated with the new agent were able to regenerate when transplanted, this was somewhat reduced at the higher dose range. Less tumor inhibition was observed in well-established tumors than those transplanted one day before.

4. Tumors from mice treated with streptovitamin A showed liquefactive changes and necrosis histologically. In the usual antitumor doses the agent does not produce a leukopenia.

# Present Status of Vancomycin Therapy of Staphylococcal and Streptococcal Infections

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Our initial laboratory and clinical studies of vancomycin\* were reported at the Antibiotic Symposium two years ago.<sup>1</sup> Promising clinical results were obtained, but phlebitis, drug fever, and renal irritation were noted in some patients with the relatively impure lots of vancomycin then available. Improvements in manufacture have since led to a more purified preparation that is much better tolerated and produces relatively few side effects. The present report is concerned with additional in vitro observations and with clinical results obtained in 32 patients treated between September, 1957, and September, 1958.

## METHODS

*Sensitivity Tests.* Concentrations of vancomycin, ristocetin, and kanamycin causing inhibition of growth of staphylococci were determined in Tryptose phosphate broth. First, 0.5 ml. amounts of a  $10^{-2}$  dilution of an overnight culture were added to equal quantities of broth containing appropriate concentrations of the antibiotic. The cultures were incubated at 37 C., and the lowest concentration of the antibiotic causing inhibition of growth by gross inspection was recorded. In some instances, the tubes were agitated, and a loopfull of broth was streaked on a blood agar plate to test for bactericidal activity. A similar method was used for streptococci, except that 5 per cent human blood was added to the broth.

*Measurement of Vancomycin Serum Concentrations.* Serum was obtained at various intervals following injections of vancomycin and was frozen and stored in sterile tubes until assays were performed. A tube dilution test was used, with macroscopic inhibition of a group A *Streptococcus* in 3 per cent blood broth as indicator. The end point was the lowest concentration of the standard or unknown in which there was no hemolysis, and there was usually no growth of the test organism when this tube was subcultured. The inoculum in each tube, as determined by plate counts, was 25,000 to 50,000 bacteria/ml., and the end point for the vancomycin standard was usually 1  $\mu\text{g.}/\text{ml.}$

*Clinical Trials.* From September 1, 1957, to September 1, 1958, 32 patients were treated with vancomycin. The antibiotic was supplied as a powder in 0.5 Gm. ampoules, and 10 ml. of sterile distilled water was added to each ampoule. This concentrated solution was diluted in 100 to 200 ml. of 5 per cent dextrose in distilled water and was administered as an intravenous infusion over a period of 20 to 30 minutes. Most patients received 1 Gm. every 12 hours for the first five to seven days and 1 Gm. daily thereafter. In no instance was more than 2 Gm. given in a 24 hour period. The duration of therapy varied from 6 to 35 days. In 16 patients the drug was given for approximately two weeks, and 9 patients received it for more than three weeks.

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\* The trade name of Eli Lilly & Co. for vancomycin is Vancocin.

TABLE I

*Relative In Vitro Activity of 3 New Antibiotics Against Recently Isolated  
Strains of Staphylococcus aureus*

Antibiotic	Total no. strains	Inhibitory concentration, $\mu\text{g.}/\text{ml.}$				
		>10	10	5	2	1
Kanamycin	54	37	8	7	1	1
Ristocetin	32	0	14	18	0	0
Vancomycin	70	0	0	11	57	2

#### LABORATORY RESULTS

Results of sensitivity tests of recently isolated strains of *Staphylococcus aureus* to kanamycin, ristocetin, and vancomycin are presented in table I. Despite the fact that both ristocetin and vancomycin have been used clinically for two years at the King County Hospital, resistant strains have not appeared. All of the 70 strains tested in the past year were inhibited by 5  $\mu\text{g.}/\text{ml.}$  of vancomycin, and only 11 required more than 2  $\mu\text{g.}/\text{ml.}$  From a comparative standpoint, ristocetin showed less activity than vancomycin, and kanamycin was less active than ristocetin.

With the large inoculum and relatively short incubation period used in these studies, bactericidal end points were considerably higher than the bacteriostatic end points recorded by observing macroscopic inhibition of growth. From a qualitative standpoint, subcultures showed that vancomycin was more bactericidal than ristocetin, and kanamycin killed fewer organisms than ristocetin. Even with vancomycin, however, a few colonies of staphylococci often grew when tubes containing 100  $\mu\text{g.}/\text{ml.}$  were subcultured.

Sixty freshly isolated strains of group A streptococci and 23 strains of *Streptococcus viridans* were inhibited by 1  $\mu\text{g.}/\text{ml.}$  of vancomycin, and there was no growth when these tubes were subcultured. In contrast, 30 strains of enterococci required 1 to 3  $\mu\text{g.}/\text{ml.}$  of vancomycin or ristocetin to inhibit growth, and many colonies were invariably present when tubes containing 10  $\mu\text{g.}/\text{ml.}$  were subcultured. These results indicate that vancomycin and ristocetin are similar to penicillin in that they are considerably less bactericidal against enterococci than against group A streptococci and *Str. viridans*. It is of interest that Romansky and Holmes<sup>2</sup> found enterococci to be considerably more susceptible to ristocetin than has been true in the present study. They have reported good results in the short-term therapy of enterococcal endocarditis with ristocetin.

Serum concentrations of vancomycin were determined in 10 of the 32 patients treated. The results were very similar to those reported in an earlier paper<sup>1</sup> and will not be repeated in detail here.

#### CLINICAL RESULTS

*Staphylococcal Infections.* The 32 patients with staphylococcal and streptococcal infections are classified according to etiology and results in table II. Additional information concerning the 30 patients with staphylococcal infections is presented in table III. All but 4 were treated at the King County Hospital, and all had severe infections. In 24 instances the patients were admitted to the hospital with underlying diseases, and antibiotic-resistant staphylococcal infections were acquired in the hos-

pital. Most patients were debilitated, and almost one-third were more than 70 years old. A partial list of their underlying illnesses is presented in table III. This background information is obviously important in understanding the therapeutic challenge presented. Despite the severity of the illnesses and the fact that 17 had staphylococcal septicemia in addition to underlying disorders, only 8 of the patients are dead at the present time. Four of these died during or shortly after vancomycin therapy, and 4 died two or more months later of unrelated illnesses.

The clinical results were particularly striking in the 17 patients with staphylococcal septicemia, in view of the high mortality associated with this disease. Thirteen of the 17 patients were cured, although 2 of these died at a later date, 1 of metastatic cancer and the other of pyelonephritis secondary to prostatic disease. At autopsy this latter patient showed evidence of healed staphylococcal endocarditis. Of the 4 patients who died during or shortly after therapy, 1 had severe third degree burns of the entire body, with anuria and agamma globulinemia; 1 died of a massive gastrointestinal hemorrhage on the sixth day of therapy, although her temperature was declining; and a third patient, 88 years old with a severe wound infection of the hip, died on the eighth day of therapy of heart failure, although the infection seemed to respond and she was afebrile. In these 3 cases the results were considered indeterminate. The fourth patient, a 71 year old woman with fever and positive blood cultures following surgery for cancer of the colon, responded well to vancomycin and was sent to a nursing home afebrile, with negative blood cultures, after two weeks of therapy. Fever returned two weeks later and she died with positive blood cultures and a relapse of staphylococcal endocarditis. Although classified as a poor result, the initial response to therapy was good in this patient, and she might have recovered if treatment had been continued for four to six weeks. Only 3 of the 17 patients with staphylococcal septicemia received vancomycin for more than 16 days, implying that short-term vancomycin therapy may be feasible in some cases, as suggested by others.<sup>2, 3</sup>

Of the 13 patients with staphylococcal septicemia who were cured, there were a number who responded after other antibiotics had failed, and, in some instances, the administration of vancomycin was associated with a dramatic drop in temperature and improvement in the clinical condition. In other instances the temperature

TABLE II  
*Clinical Results in 32 Patients Treated with Vancomycin*

Diagnosis	Total no. patients	Response to therapy		
		Good	Indeterminate	Poor
Staphylococcal septicemia	17			
Narcotic injections	3	3		
Tooth extraction	1	1		
Aspiration pneumonia	2	2		
Infected cutdown	5	4	1	
Wound infection	2		1	1
Genitourinary disorders	3	3		
Infected burn	1		1	
Other staphylococcal infections	13			
Wound infections	5	5		
Aspiration pneumonia	6	6		
Osteomyelitis	2	2		
Streptococcal infections	2			
Enterococcal sepsis	1	1		
<i>Streptococcus viridans</i> endocarditis	1	1		

TABLE III  
Pertinent Details Concerning 30 Patients with Severe Staphylococcal Infections

Data	Number of patients
Infection acquired in hospital	24
Septicemia	17
More than 50 years old	23
More than 70 years old	12
Associated illnesses	
Carcinoma	5
Hip or leg fracture	7
Valvular heart disease	2
Gastrointestinal bleeding	2
Alcoholism	7
Narcotics addiction	3
Pancreatitis	1
Ulcerative colitis	2
Patients who have died	8
During or shortly after vancomycin therapy	4
Died later of underlying illness	4

declined more slowly, but blood cultures became negative and clinical improvement was evident within a few days. Because of limitations of space, detailed information and case reports concerning the patients with staphylococcal septicemia will be presented elsewhere.

Two patients with chronic osteomyelitis showed a definite improvement on vancomycin therapy following failure to respond to other antibiotics. In both instances, signs of inflammation subsided and there was a complete cessation of drainage from sinus tracts. In both instances, however, a relapse occurred several months later. These results are comparable to those obtained a decade ago with penicillin therapy, and they demonstrate the difficulties in bringing about a permanent cure in osteomyelitis unless definitive surgery can be performed in conjunction with antibiotic therapy. The response in patients with severe wound infections and staphylococcal pneumonia without bacteremia was good, and in most of these 11 cases, the infecting *Staphylococcus* was highly resistant to other antibiotics. All recovered from the staphylococcal infection, but 2 died later, one from a cerebral thrombosis and the other from pulmonary insufficiency following a lobectomy.

*Streptococcal Infections.* A patient with bacterial endocarditis due to *Str. viridans* had a severe reaction following an initial dose of penicillin and was therefore treated with vancomycin. There was an immediate response, with negative blood cultures overnight, and vancomycin was given for only two weeks. This patient has been followed for almost a year with no relapse. Another patient developed enterococcal septicemia five months following removal of a bladder cancer. He received vancomycin for one month, during which he became afebrile and blood cultures were negative. He pursued a downhill course, however, and died three weeks later. At autopsy enterococcal endocarditis was found, but culture of the heart valves was sterile. There were two large abscesses in the spleen, and enterococci were cultured from these. Thus, although there was a good response to vancomycin therapy, the presence of the underlying carcinoma and of splenic abscesses probably led to the fatal termination.

#### SIDE EFFECTS

With the relatively pure antibiotic powder available during the past year, ad-

ministration of vancomycin has been associated with a striking lack of toxicity and side effects. Phlebitis of a mild degree occurred in some instances, but in most patients this caused very little difficulty. There seemed to be little more irritation of veins than was present when glucose solutions were administered separately. In a number of patients vancomycin was administered through femoral or caval catheters, with no apparent deleterious effects.

Nephrotoxicity has not been noted during the past year. Repeated examinations of the urinary sediment and blood urea nitrogen determinations were made before and after therapy in 20 of the 32 patients treated. In no instance was there evidence of renal irritation, and in a number of cases there was a decline in the blood urea nitrogen while the patient was on therapy. The manifestations of renal irritation previously noted appear to have been eliminated by the preparations of vancomycin that are now available.

Two patients noted fever, itching rash, and ringing in the ears when vancomycin therapy was started. In both instances the reaction was alleviated by the administration of antihistamines. One patient received chlorprophenpyridamine maleate in the intravenous infusion for several days, and there were then no further reactions when this was stopped. The other patient received a month's course of vancomycin therapy six months later, and this time no reaction occurred. In another patient, not included among the present group, a rash occurred with vancomycin, and this patient was treated with ristocetin without side effects. We have similarly noted patients who developed a rash with ristocetin but tolerated vancomycin without difficulty. In all 3 of the cases of drug hypersensitivity, prolonged antibiotic therapy had been given in the past for osteomyelitis, including streptomycin. This suggests that prolonged administration of streptomycin may lead to cross sensitization with vancomycin, but this is purely speculative at present.

One patient in the present series developed deafness. He had received neomycin parenterally for over a month prior to admission and had slightly impaired hearing at that time. During the next two weeks he became totally deaf and has remained so, although he responded dramatically to vancomycin therapy. It is probable that neomycin was responsible for deafness in this case, although we cannot exclude the possibility that vancomycin played a role. There was no hearing loss apparent in any of the 31 other patients, although deafness has been described in association with very high vancomycin serum concentrations.<sup>3</sup>

#### DISCUSSION

From the present studies it appears that vancomycin is probably the best single agent available at present for the treatment of severe staphylococcal infections. In vitro, vancomycin is more active and has greater bactericidal power than two other new staphylococcal antibiotics, kanamycin and ristocetin. Other important advantages of vancomycin are the high, sustained blood levels that are readily attained and the fact that vancomycin-resistant staphylococci have not been encountered after using this antibiotic clinically for more than two years. For milder staphylococcal infections, the combination of erythromycin and chloramphenicol has proved to be adequate in the experience of many investigators. In the present series, however, patients with severe infections have responded to vancomycin therapy after this combination has failed. Of the other antistaphylococcal drugs available, oleandomycin, spiramycin, and nitrofurantoin are definitely weaker in action and should not, in our opinion, be used in the therapy of severe staphylococcal sepsis. Bacitracin

is bactericidal, but its toxicity precludes giving doses that will produce high blood levels. Neomycin is highly effective, but because of nephrotoxicity and a high incidence of deafness its use is not warranted. Novobiocin is very active *in vitro*, but its action is antagonized by serum, the incidence of skin rash is quite high, and antibacterial resistance develops rapidly. These statements comparing vancomycin with other antistaphylococcal agents apply particularly when the infecting organism is resistant to penicillin, tetracyclines, and streptomycin, which is the case in the majority of hospital acquired infections.

The chief disadvantage of vancomycin therapy is the necessity of administering it in an intravenous infusion once or twice daily. In most instances this is not a serious problem, since patients with severe staphylococcal infections usually need intravenous fluids to provide adequate hydration and nutrition. In a number of patients with poor veins, vancomycin has been administered through femoral or caval catheters without any apparent untoward effects. The side effects associated with early lots of vancomycin, namely, phlebitis, drug fevers, and renal irritation, have been largely eliminated by the purer preparations now available. With few exceptions, side effects have not interfered with the administration of vancomycin during the past year.

Because of difficulties in administration, vancomycin is obviously not indicated for the therapy of all staphylococcal infections. In our opinion, vancomycin should be given whenever there is a possibility of dissemination of the infection. When multiple abscesses have formed throughout the tissues and particularly when there are large abscesses, antibiotics, including vancomycin, usually fail to eradicate the infection. Deep seated abscesses are difficult to localize and often impossible to drain, and in these instances a fatal termination is inevitable. In a number of instances we have withheld vancomycin until too late, hoping to eradicate a moderately severe staphylococcal infection with other antibiotics, such as chloramphenicol and erythromycin. Our tendency at present is to use vancomycin more frequently and to start it earlier, particularly in debilitated patients with hospital acquired infections.

#### SUMMARY AND CONCLUSIONS

Although vancomycin and ristocetin have been used clinically for over two years at the King County Hospital, no vancomycin-resistant staphylococci have been encountered. Seventy recently isolated strains were inhibited by 5  $\mu\text{g.}/\text{ml.}$  of vancomycin, and only 11 grew in the presence of 2  $\mu\text{g.}/\text{ml.}$  Comparative studies showed that ristocetin and kanamycin were definitely less active than vancomycin *in vitro*. Vancomycin and ristocetin were considerably less bactericidal against enterococci than against group A streptococci and viridans streptococci.

Despite promising clinical results, the relatively impure lots of vancomycin available two years ago produced phlebitis, drug fever, and renal irritation in some cases. The clinical material now available is much better tolerated and produces very few side effects. Thirty-two patients with severe staphylococcal and streptococcal infections were treated with vancomycin from September, 1957, to September, 1958. Seventeen had positive blood cultures, and therapy ranged in duration from 6 to 35 days. Results were, in general, excellent and were often dramatic in instances in which other antibiotics had failed. Vancomycin is probably the best drug now available for the treatment of severe staphylococcal infections caused by penicillin-resistant organisms.

The vancomycin utilized in this study was furnished by Eli Lilly & Co.

## BIBLIOGRAPHY

1. KIRBY, W. M. M., AND DIVELBISS, C. L.: Vancomycin: clinical and laboratory studies. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 107.
2. ROMANSKY, M. J., AND HOLMES, J. R.: Successful short-term therapy of enterococcal and staphylococcal endocarditis with ristocetin—seven patients. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 187.
3. GERACI, J. E.; HEILMAN, F. R.; NICHOLS, D. R., AND WELLMAN, W. E.: Antibiotic therapy of bacterial endocarditis. VII. Vancomycin for acute micrococcal endocarditis. Preliminary report, *Proc. Staff Meet., Mayo Clin.* 33:172, April 2, 1958.

# The Clinical Evaluation of Vancomycin in Treatment of Multi-Antibiotic Refractory Staphylococcal Infections

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While the numbers of therapeutic agents capable of inhibiting staphylococcal growth have been steadily increasing, the management of these infections continues to be a major medical problem. In addition to laboratory and clinical evidence of antistaphylococcal action, the assessment of a new antibiotic should include an evaluation of its ability to check these infections after other drugs have failed. This stringent test also permits assignment of relative clinical merit for the various antibiotics.

Laboratory and clinical studies have already indicated the potential value of vancomycin\* in treatment of staphylococcal disease.<sup>1,2</sup> The present report deals with the usefulness of vancomycin in therapy of staphylococcal infections that have progressed in the face of systemic administration of two or more antibiotics chosen by in vitro test.

## MATERIALS AND METHODS

The 10 patients included in this study were hospitalized at the Jackson Memorial Hospital. Their diseases were: pneumonia, meningitis, parotitis, bacteremia, osteomyelitis, and arthritis (table I). Staphylococcal infection was proved in each case by isolating coagulase-positive organisms from the local site as well as by the typical clinical picture.

Indications for the use of vancomycin were: clinical and bacteriological evidence of continuing staphylococcal infection after a minimum of five days' systemic treatment with two or more antibiotics that had been chosen by in vitro test. Choice of initial antibiotic therapy was based on demonstration of bacterial sensitivity by the disc method.

An independent clinical opinion of the patient's condition was obtained from his physician. Every patient in the study was considered to be critically ill. In 9 cases the infection appeared life-threatening.

Vancomycin was administered intravenously in physiological saline or dextrose solutions as the sole systemic antibiotic. A priming dose of 1 to 2 Gm. was generally given at the outset. Thereafter, 0.5 Gm. quantities were injected at intervals of six to eight hours for a 7 to 16 day period. Two patients received oral antibiotics after the course of vancomycin.

All patients were seen daily. Hearing ability was tested grossly wherever possible. Biochemical tests of hepatic and renal functions were done at weekly intervals; complete blood counts were done twice weekly.

Exudate from the infected site was cultured as available. Cultures of the blood and nasopharynx or trachea were made twice weekly.

Staphylococci (both coagulase-positive and -negative) that were recovered during the course of vancomycin therapy were tested for vancomycin resistance. For

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\* The trade name of Eli Lilly & Co. for vancomycin is Vancocin.

TABLE I  
*Patients Treated With Vancomycin*

Patient	Age, yr.	Staphylococcal infection	Staphylococcal sensitivity with first therapy	Initial therapy	Durat. initial Staphylococcal therapy, days	Vancomycin therapy			Comment	
						Gm.	Days	Result		
1. R. Ha.	13	Pneumonia, empyema, osteomyelitis	*	Penicillin Streptomycin Novobiocin Chloramphenicol	19 5 13 13	Penicillin Streptomycin Novobiocin Chloramphenicol	21.5	14	Cured	Histamine-like side reaction
2. S. Da.	51	Arthritis	Novobiocin Chloramphenicol Bacitracin Neomycin	Novobiocin Chloramphenicol (Bacitracin and neomycin injected into joint)	16 40 35	Novobiocin Bacitracin Neomycin	23	14	Cured	Rash on 10th day of vancomycin
3. L. Ha.	69	Parotitis, bacteremia	Chloramphenicol Erythromycin	Chloramphenicol Erythromycin	10 10	Chloramphenicol Erythromycin	16.5	10	Cured	2 week course of novobiocin given after completion of vancomycin
4. D. Ma.	75	Osteomyelitis, bacteremia	Chloramphenicol Erythromycin	Chloramphenicol Erythromycin	7 6	Chloramphenicol	22.0	16	Cured	Thrombophlebitis on 4th day; rash on 15th day of vancomycin
5. R. Bo.	53	Massive parotitis	Chloramphenicol Erythromycin	Chloramphenicol Erythromycin	5 5	Erythromycin	21.5	10	Improved; incision on 7th day	
6. D. Si.	56	Meningitis, ophthalmitis	Chloramphenicol Erythromycin	Chloramphenicol Erythromycin	6.5 6.5	Chloramphenicol Erythromycin	29	14	Improved; aspiration of retro-orbital pus on 7th day	
7. J. Is.	69	Pneumonia	Penicillin Streptomycin Tetracycline Chloramphenicol Erythromycin	Penicillin Streptomycin Chloramphenicol Erythromycin	6 6 5 4	Chloramphenicol Erythromycin	15	10	Improved; tracheostomy reopened	4 day course of chloramphenicol and erythromycin after completion of vancomycin
8. M. LaG.	45	Multiple abscesses, bacteremia	Novobiocin Chloramphenicol	Novobiocin Chloramphenicol Erythromycin	36 23	Novobiocin Chloramphenicol Erythromycin	7.0	4	Died with continuing infection	In uremia
9. J. McC.	55	Pneumonia, bacteremia	Chloramphenicol Erythromycin	Chloramphenicol Erythromycin	8 8	Chloramphenicol Erythromycin	17	6	Died with continuing infection	In shock; on steroids and norepinephrine
10. D. Da.	62	Parotitis, pneumonia	Novobiocin Erythromycin	Novobiocin Erythromycin	5 7	Novobiocin	19	10	Died with continuing infection	Diabetic; recently in coma and acidosis

\* Not available, transferred from another hospital.

TABLE II  
*Vancomycin Sensitivities of Staphylococci Isolated during Treatment*

Patient	Source	Day of vancomycin treatment	Bacteriostatic end point, $\mu\text{g.}/\text{ml.}$	Bactericidal end point, $\mu\text{g.}/\text{ml.}$
1. R. Ha.	Nasopharynx	8	1	16*
	Nasopharynx	12	1	1
4. D. Ma.	Pharynx	7	1	8
	Pharynx	14	1	8
	Pharynx	14	<1	8
5. R. Bo.	Parotid	0	1	1
	Trachea	3	<1	4
	Nose	7	1	2
	Parotid	7	<1	1
6. D. Si.	Nose	0	1	2
	Nose	6	2	8
	Trachea	15†	<1	<1
	Trachea	27†	<1	<1
7. J. Is.	Trachea	2	1	1
	Trachea	14	1	4
8. M. LeG.	Pharynx	1	<1	2
	Blood	4	<1	2
10. D. Da.	Trachea	0	1	2
	Nose	3	1	1
	Nose	8	1	2
	Nose	11	1	2

\* Not tested against higher concentrations. † Vancomycin discontinued at fourteenth day.

each organism, 1 ml. of a  $10^{-4}$  dilution of an 18 hour broth\* culture was added to 1 ml. of varying quantities of vancomycin in broth, so that the final concentrations were 16, 8, 4, 2, and 1  $\mu\text{g.}/\text{ml.}$

After incubation for 48 hours at 37 C., turbidity in the highest concentration of vancomycin was recorded as the bacteriostatic end point. A loopful of each tube that did not show growth was subcultured onto a blood or plain agar plate, and these plates then were incubated at 37 C. for an additional 48 hours. Growth on subculture from the highest vancomycin concentration was taken as the bactericidal end point. Growth was confirmed by microscopic examination.

#### RESULTS

Of 10 patients in the study, staphylococcal infection was cured by vancomycin alone in 4 (R. Ha., D. Ma., S. Da., and L. Ha.). Previous surgical drainage in 3 of these (R. Ha., D. Ma., and S. Da.) had failed to eradicate the infection. In 3 other patients (J. Is., D. Si., and R. Bo.), the improvement occurred with vancomycin treatment and surgical drainage 1 to 10 days later.

In none of these successfully treated patients was the previous failure of antibiotic therapy associated with the development of complete in vitro resistance by the infecting bacteria (table I). Thus, these infections had been refractory to prior treatment for reasons other than the evolution of antibiotic-resistant organisms.

Three patients died of the infection despite all therapeutic efforts (M. LeG., J. McC., D. Da.). In each case, serious underlying medical disease was present.

Bacteriostatic concentrations of vancomycin for the 21 staphylococcal isolates were 2  $\mu\text{g.}/\text{ml.}$  or less. Bactericidal levels were 8  $\mu\text{g.}/\text{ml.}$  or less in 20. No development of vancomycin-resistance was observed in patients who died or patients who were cured with the drug (table II).

\* Trypticase soy broth (Baltimore Biological Laboratories) was used throughout.

TABLE III  
*Untoward Reactions to Vancomycin*

Patient	Reaction	Day of vancomycin treatment	Comment
1. R. H.	Hives, fever, and diffuse erythema	1	Drug continued for two weeks; further reactions prevented with antihistamines
2. S. Da.	Diffuse maculopapular skin eruption	10	Drug continued for four days; skin reaction worsened
3. D. Ma.	Diffuse maculopapular skin eruption	15	Drug continued for one day; skin reaction worsened
3. D. Ma.	Thrombophlebitis	4	Occurred at site of intravenous clisis continued for four days

Side effects (table III) were limited to maculopapular eruptions of the face and body in 2; hives, erythema, and fever in 1 (easily controlled with antihistamines); and thrombophlebitis at the site of a prolonged intravenous clisis in 1.

Case reports of the 10 patients follow.

*Case 1.* R. Ha., a 13 year old white boy, at first contracted pneumonia. Despite treatment with penicillin, streptomycin, and tetracycline, empyema and tension pneumothorax ensued. One million to 6,000,000 units of penicillin a day for 19 days, tetracycline, 1 Gm. a day for five days, and streptomycin, 1 Gm. a day for five days, were administered intramuscularly. Thereafter chloramphenicol, 1 to 3 Gm. a day, was given for 13 days, novobiocin, 2 Gm. a day for 13 days, and oleandomycin-tetracycline (Signemycin\*) mixture, 2 Gm. a day for 11 days, all orally.

The patient was then transferred to the Jackson Memorial Hospital. Clinical and roentgen-ray studies revealed a pyopneumothorax of the right chest and osteomyelitis of the right humerus and femur. *Staphylococcus aureus* was recovered from the empyema fluid and urine. The organism was sensitive to penicillin, streptomycin, chloramphenicol, and novobiocin. Closed catheter drainage of the right chest was then instituted, but the patient's temperature continued to range from 102 to 103 F. After four days of empyema drainage with no clinical improvement, vancomycin was started.

The patient received 21.5 Gm. of vancomycin over a 14 day period. The empyema cleared (closed chest suction was continued), the lung re-expanded, and the osteomyelitis cleared. He became afebrile, gained weight, and recovered completely.

Side effects were diffuse erythema, hives, and fever, which started 30 seconds after the first vancomycin dose. This was easily controlled and subsequently prevented with antihistamines.

Serial studies of liver function, renal function, and blood count revealed no abnormalities. This patient is considered a vancomycin cure.

*Case 2.* S. Da., a 51 year old white man, was admitted because of persistent staphylococcal arthritis of the right knee. The patient had had rheumatoid arthritis for 15 years for which he had received corticosteroid therapy for eight years.

His first signs of septic arthritis were severe pain and swelling of the right knee. The corticosteroids were stopped. Cultures revealed *Staph. aureus* sensitive to novobiocin, chloramphenicol, bacitracin, and neomycin. The knee was surgically drained and marsupialized. The patient developed empyema of the right chest from which staphylococci were cultured as well. The empyema was drained by thoracotomy. Antibiotic therapy for the arthritis was as follows: erythromycin, 2 Gm. a day orally for 24 days; chloramphenicol, 2 Gm. a day orally for 40 days; and novobiocin, 1 Gm. a day orally for 16 days. In addition, antibiotics were injected into the knee as follows: neomycin, 1 Gm. a day, and bacitracin, 100,000 units a day, for five weeks. Despite these medications the pain and drainage continued for four months. Cultures of the knee were repeatedly positive for *Staph. aureus* during this four month period, although the staphylococci disappeared from the empyema fluid. The chronic empyema has persisted to date.

The patient was then given a two week course of vancomycin, total dosage being 23 Gm.

\* The trade name of Chas. Pfizer & Co. for the combination of oleandomycin and tetracycline is Signemycin.

With this, a remarkable improvement occurred and the draining and pain subsided. Cultures of the knee became negative and the excess synovial fluid disappeared.

While roentgenograms had revealed evidence of osteomyelitis around the bones of the knee within one month of the onset of septic arthritis, there was no evidence of extension of the process up or down the long bones thereafter.

On the tenth day of vancomycin therapy, a diffuse maculopapular eruption appeared over the trunk and there was periorbital and facial edema. Vancomycin was continued, however, for the next four days and the rash worsened. After cessation of the vancomycin, the rash faded in approximately one week.

There was no evidence of renal, hepatic, or bone marrow derangement by vancomycin. This case is regarded as a cure of staphylococcal arthritis by vancomycin. Further follow-up of the osteomyelitis is necessary.

*Case 3.* L. Ha., a 69 year old white man, was admitted in a comatose state with severe dehydration and right hemiplegia. Parotitis and bacteremia, both due to *Staph. aureus*, occurred during hospitalization. The organism was sensitive to chloramphenicol and erythromycin. The patient was treated with these two drugs for 10 days, 2 Gm. a day of each orally and parenterally. Despite this, the temperature remained elevated and the salivary glands continued to drain purulent material containing *Staph. aureus*. In addition, fresh crops of furuncles developed suggesting continuing bacteremia, and diarrhea ensued with *Staph. aureus* in the feces.

A 10 day course of 16.5 Gm. of vancomycin was given with dramatic improvement. The salivary gland infection disappeared, the patient's temperature became normal, and the diarrhea ceased. No side effects to vancomycin were noted. No evidence of hepatic, renal, or bone marrow dysfunction was observed.

After clinical and bacteriological cure with vancomycin, a two week course of novobiocin, 1.5 Gm./day, was administered.

*Case 4.* D. Ma., a 75 year old previously healthy white man, fractured the right patella in an automobile accident. One week later fragments of the patella were surgically removed. Four days after surgery the temperature rose to 101 F. and furunculosis was noted. Cultures from the furuncles revealed *Staph. aureus*, sensitive to chloramphenicol and erythromycin. Thereafter, there were daily elevations in temperature to between 101 and 102 F. Culture of the surgical wound revealed *Staph. aureus*, sensitive to chloramphenicol and erythromycin. The knee was reopened and 50 ml. of purulent material was aspirated, after which *Staph. aureus* bacteremia and temperature elevation to 106 F. occurred. The first antibiotic therapy consisted of chloramphenicol, 3 Gm. a day intramuscularly for seven days, and erythromycin, 3 Gm. a day intramuscularly for six days. In spite of this, the patient's temperature continued to range between 101 and 103 F. Wound cultures during this therapy continued to be positive for staphylococci. The patient was then started on a 15 day course of 22 Gm. of vancomycin and had progressive improvement with subsidence of both local and general symptoms and healing of the knee.

On the fourth day of vancomycin treatment, thrombophlebitis took place at the site of an intravenous clysis which had been continued for four days. On the fifteenth day of vancomycin treatment, the patient had a generalized maculopapular rash, which lasted for two weeks.

There was no evidence of hepatic, renal, or bone marrow impairment. This case is regarded as cured by vancomycin.

NOTE. Intermittent soaks of bacitracin-neomycin solution were applied to the wound during both the chloramphenicol and erythromycin therapy and the vancomycin therapy.

*Case 5.* R. Bo., a 53 year old white man, was admitted in a semicomatose state with temperature of 103.4 F. and signs of a right lower lobe pneumonia. He was known to be a chronic alcoholic.

He was given chloramphenicol and erythromycin, 1 Gm. a day of each, intramuscularly, for five days because of *Staph. aureus* in the sputum. In spite of this, a 3 by 5 cm. mass appeared in the right face anterior to the ear, draining pus into the mouth via Stensen's duct. The white blood count ranged between 18,000 and 34,000/cu. mm. Culture of the purulent material revealed *Staph. aureus*, sensitive to chloramphenicol and erythromycin. While chloramphenicol and erythromycin therapy was continued, the mass increased in size, extending from the angle of the jaw to within 3 cm. of the nostril. The patient became desperately ill, with increasing mental confusion and cyanosis.

Vancomycin therapy of 21.5 Gm. over 10 days was carried out. There was considerable improvement with decrease in the abscess size, decrease in temperature, and increase in responsiveness of the patient. After seven days of vancomycin treatment, incision and drainage of the parotid abscess was carried out. Only two isolated pockets of purulent material remained in the gland.

Forty-eight hours after this surgical procedure the patient became comatose and died. Autopsy revealed carcinoma of the lung, carcinoma of the thyroid, metastatic carcinoma to the left cerebellar hemisphere, and subsiding parotitis. There was no evidence of damage to liver, kidney, or bone marrow while on vancomycin.

This case is considered as one in which improvement of parotid abscess occurred due to vancomycin and subsequent surgical drainage.

*Case 6.* D. Si., a 56 year old white man, a chronic alcoholic, had been hospitalized elsewhere for six weeks for the treatment of staphylococcal meningitis and orbital cellulitis after injury to the right eye. During this time he had been treated with erythromycin, chloramphenicol, and tetracycline, and he had improved considerably. After cessation of drugs he relapsed, convulsing and becoming comatose.

Lumbar puncture revealed 8300 cells/cu. mm., 96 per cent polymorphonuclear cells, and the spinal fluid sugar was 15 mg./100 ml. Culture of the spinal fluid revealed *Staph. aureus*, sensitive to chloramphenicol and erythromycin, but resistant to penicillin.

The patient was treated with 2 Gm. each of chloramphenicol and erythromycin per day, intravenously, for 6.5 days. He also received 10,000,000 units of penicillin intravenously for three days. Despite these medications, three lumbar punctures in the next six days yielded *Staph. aureus* on culture. The spinal fluid cell count after six days of this therapy was 9650/cu. mm., with the spinal fluid sugar less than 10 mg./100 ml. A course of 29 Gm. of vancomycin was then given over 14 days. On the day vancomycin was started, the spinal fluid culture was positive for *Staph. aureus*, as it was on the next two days. Thereafter, all six spinal fluid cultures taken in the next 16 days were negative.

On the tenth day of vancomycin therapy, a few drops of pus were removed by aspiration of the retro-orbital space. Culture revealed *Staph. aureus*.

The patient made a dramatic recovery prior to the retro-orbital space aspiration, with a drop in white blood cells in the spinal fluid from 10,000 to 500/cu. mm. and a rise in the spinal fluid sugar from less than 10 to 60 mg./100 ml. Clinically, the improvement was also dramatic. He awakened and became responsive. By the time of his hospital discharge he was able to walk (with aid) and feed himself.

No side effects of vancomycin were observed. Hepatic and renal functions were intact and there was no evidence of bone marrow depression. This case is considered as improved by vancomycin and retro-orbital space aspiration.

*Case 7.* J. Is., a 69 year old white man, was admitted in coma with signs of a cerebrovascular accident. After 10 days in the hospital, clinical and roentgen-ray evidence of pneumonia in the left lower lobe became apparent. Culture from the trachea via a tracheostomy revealed *Staph. aureus*, sensitive to tetracyclines, penicillin, streptomycin, chloramphenicol, and erythromycin. The patient was treated for six days with 800,000 units of penicillin a day and 1 Gm. of streptomycin a day intramuscularly; next, 2 Gm. of erythromycin a day for four days orally; then, 1 Gm. of chloramphenicol a day for five days orally. Blood levels of these antibiotics were undoubtedly much higher than are usually attained with this dosage as the patient's renal function was impaired. Blood nonprotein nitrogen values were 70 to 80 mg./100 ml. Tracheal aspirations continued to reveal *Staph. aureus*. The tracheostomy site was allowed to close for four days. Fever and roentgen-ray evidence of pneumonia persisted.

The patient was then started on a 10 day course of 15 Gm. of vancomycin, and the next day the tracheostomy site was reopened. There was decrease in dyspnea, fever, and considerable improvement of both roentgen-ray and physical signs of pneumonia. After cessation of vancomycin, a course of chloramphenicol and erythromycin, 2 Gm. a day of each orally, was administered for four days. The patient had had a series of vascular emboli resulting in gangrene of the right arm and the left leg. After surgery for the gangrene he died.

Autopsy revealed multiple systemic emboli, several pulmonary ones, and healing bronchopneumonia. The improvement of the staphylococcal bronchopneumonia is attributed to vancomycin and to reopening of the tracheostomy.

*Case 8.* M. LeG., a 45 year old white woman, was transferred from another hospital where she had been treated for six weeks for multiple staphylococcal skin abscesses, congestive heart

failure, and diabetes mellitus. Two Gm. a day of novobiocin had been given orally for 36 days, in addition to other systemic and local antibiotics. Multiple draining abscesses were present over the chest, neck, back, and thighs; some had been surgically incised. *Staph. aureus* was recovered from the abscesses, sensitive to chloramphenicol, erythromycin, and novobiocin. *Staph. aureus* in the blood cultures was sensitive to chloramphenicol and novobiocin but not to erythromycin.

Because of lack of improvement with novobiocin, chloramphenicol and erythromycin, 2 Gm. a day of each orally, was given for 18 days. The patient made an excellent response. Blood cultures became negative, her temperature returned to normal, and she became alert and responsive for the first time during this hospitalization. At this point, systemic antibiotics were stopped. Ten days later temperature elevation recurred and blood cultures again became positive for *Staph. aureus*. Erythromycin and chloramphenicol, 3 Gm. a day intramuscularly of each, were administered again for five days. Despite this, blood cultures continued to yield *Staph. aureus*.

Vancomycin was then started, a total of 7 Gm. being given over four days. Her febrile state continuing, the patient became progressively unresponsive, went into shock, and died.

Autopsy revealed subcutaneous abscesses in the chest, psoas abscess, breast abscesses, lung abscesses, renal abscesses, and the granular contracted kidneys of chronic pyelonephritis.

The patient had had uremia in the last three weeks of life. Blood urea nitrogen concentrations were 40 to 100 mg./100 ml. However, in view of the continuing infection, this case is regarded as a vancomycin failure.

*Case 9.* J. McC., a 55 year old white man, was admitted from a nursing home with fever and respiratory distress. Chest roentgenogram revealed right lower lobe pneumonia. *Staph. aureus* (coagulase-negative) was cultured from the blood. Chloramphenicol and erythromycin, 1 to 2 Gm. of each intramuscularly, was given daily for eight days with no improvement. A tracheostomy was performed to facilitate aspiration of pulmonary secretions. Cultures of the tracheal exudate demonstrated coagulase-positive *Staph. aureus*, sensitive to chloramphenicol and erythromycin. The patient then went into shock; norepinephrine and hydrocortisone were given continuously.

At this point vancomycin was started, 17 Gm. being given in six days. Clinical and bacteriological evidence of infection persisted. On the sixth day of vancomycin therapy, the patient had a massive gastrointestinal hemorrhage and died. Postmortem examination was not done.

In view of the continuing infection, this case is regarded as a vancomycin failure despite death from hemorrhage.

*Case 10.* D. Da., a 62 year old white woman, was brought to the hospital in an unconscious state. Clinical and laboratory findings revealed the patient to be in diabetic acidosis and coma. On the third day of hospitalization, after appropriate fluid, electrolyte, and insulin therapy, she became alert and responsive.

On the eighth day swelling and tenderness of the left submandibular region developed and extended to involve the parotid and right submandibular glands. Temperature elevation to 103 F. occurred, and shortly thereafter, left lower lobe pneumonia developed.

Cultures of the submandibular glands revealed staphylococci, sensitive to erythromycin and novobiocin. The patient received 1.25 Gm. of erythromycin intravenously per day for seven days and 1 Gm. of novobiocin intravenously per day for five days. Despite these antibiotics there was no clinical improvement, and sputum cultures continued to be positive for staphylococci.

A 10 day course of 19 Gm. of vancomycin was administered. There was no significant clinical improvement, and on the tenth day she died. Autopsy revealed bacterial bronchopneumonia, severe bilateral tracheobronchitis, coronary arteriosclerosis, and myocardial fibrosis.

This case represents failure of vancomycin therapy.

#### DISCUSSION

The cure or improvement by vancomycin of 7 of 10 staphylococcal infections that had been progressing despite therapy with two or more theoretically effective antibiotics clearly indicates the value of this new drug. It is especially noteworthy that the infection had appeared to be life-threatening in 9 of these patients.

Three of four outright cures involved infections of bones and joints (patients

R. Ha., D. Ma., S. Da.). Further study of vancomycin in treatment of staphylococcal osteomyelitis and arthritis seems to be warranted.

Since failure of initial antibiotic therapy was not associated with the development of complete in vitro resistance by the infecting staphylococci, the reasons for vancomycin success must be sought elsewhere. Explanations of its clinical efficacy may lie in: possible increased penetration of the infected site, possible accumulation of drug resulting in high body concentrations, and/or pronounced bactericidal effect.

Vancomycin cures could not be attributed to surgical or supportive measures. Vancomycin failures were not due to resistant bacteria.

All three patients (D. Da., M. LeG., J. McC.) who died of their infection had serious underlying medical diseases or metabolic disorders. One had recovered from diabetic coma and acidosis less than one week before the onset of the staphylococcal infection. Another was in shock and being maintained on norepinephrine and hydrocortisone, and the third was in uremia. The accelerating effects of metabolic derangement on staphylococcal infections have been experimentally demonstrated by Smith and Dubos<sup>3</sup> and these factors may well have contributed to the fatal outcome.

The failure of prior bactericidal drug therapy in R. Ha. (penicillin: 1 to 6 million units a day for 19 days; streptomycin: 1 Gm. a day for five days; and novobiocin: 2 Gm. a day for 13 days), S. Da. (novobiocin: 1 Gm. a day for 19 days; plus intra-articular injections of bacitracin: 100,000 units a day; and neomycin: 1 Gm. a day for five weeks), and J. Is. (penicillin: 800,000 units a day for six days; streptomycin: 1 Gm. a day for six days; erythromycin: 2 Gm. a day for four days) needs explanation. Since antibiotic-resistant organisms did not evolve, concentrations of these drugs at the site of infection must have been inadequate.

As far as side effects are concerned, 10 patients comprise too small a group for statistical evaluation. One of the reactions was clearly histamine-like, and it may be anticipated that further purification of the drug will prevent this. The two maculopapular skin eruptions that occurred took place on the tenth and fifteenth days, respectively, of treatment. It is possible that shorter courses might avoid this. The single episode of fever and thrombophlebitis can better be attributed to prolonged intravenous clisis than to antibiotic.

Both the bactericidal effect of vancomycin at clinically attainable concentrations and the absence of development of staphylococcal resistance during therapy are consonant with previous reports.<sup>2</sup> These findings, in addition to the absence of cross resistance with other antibiotics, suggest that vancomycin will have an important place in the treatment of severe staphylococcal infections.

#### SUMMARY

A clinical study of vancomycin therapy in patients selected because of continuing staphylococcal infection despite administration of two or more antibiotics chosen by in vitro test is described. Seven of 10 patients were treated successfully. No development of vancomycin-resistant staphylococci was observed.

#### BIBLIOGRAPHY

1. GERACI, J. E.; HEILMAN, F. R.; NICHOLS, D. R.; WELLMAN, W. E., AND ROSS, G. T.: Some laboratory and clinical experiences with a new antibiotic, vancomycin. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, pp. 90-106.
2. GERACI, J. E.; HEILMAN, F. R.; NICHOLS, D. R., AND WELLMAN, W. E.: Antibiotic therapy of bacterial endocarditis. VII. Vancomycin for acute micrococcal endocarditis. Preliminary report, *Proc. Staff Meet., Mayo Clin.* 33:172-181, 1958.
3. SMITH, J. M., AND DUBOS, R.: The effect of nutritional disturbances on the susceptibility of mice to staphylococcal infections, *J. Exper. Med.* 103:521-530, 1956.

# A Critical Clinical Evaluation of Kanamycin in Severe Infections

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In spite of the availability of a number of potent and generally effective antibiotic drugs, the clinician is still occasionally faced with perplexing situations in the management of infectious diseases. The purpose of this report is to present the results obtained with kanamycin in a group of patients with a wide variety of severe infections. Our plan in preparing the report was to determine whether or not kanamycin offers advantages over presently available antibiotics and thus deserves a position of significance in the present scheme of antibiotic therapy.

In order to accomplish our stated purpose, careful selection of case material was necessary to permit a critical evaluation of the therapeutic result. Many kanamycin-treated patients were excluded from this report because they did not represent a serious challenge to the drug or because ancillary measures, such as surgical treatment, obscured the response to kanamycin.

## METHODS

Patients were selected for this study in two ways: those who had not received prior drug therapy and in whom available antibiotics could be anticipated to produce an unsatisfactory result on the basis of past experience with the involved organism and those who had an unsatisfactory response to other antibiotics. As noted in the introduction, patients included in this study all had severe, life-threatening infections, and no patients were included in whom surgical measures constituted an important feature of management.

In all cases numerous bacteriological studies were made before, during, and after treatment; in vitro sensitivity studies were performed in almost all cases. Biological blood levels of kanamycin were determined in 1 patient.

Kanamycin was administered intramuscularly in a dosage of 0.5 Gm. every eight hours, although 1.0 Gm. every eight hours was frequently used. The duration of kanamycin therapy was determined by the type of infection, clinical response, and the nature of the infecting organism. The response to therapy was graded as excellent, good, or poor. Deaths constituted a separate category.

Toxicity was appraised by manifestations of local, auditory, or vestibular effects; renal toxicity was evaluated by urinalyses and blood urea nitrogen determinations twice weekly. A percutaneous renal biopsy and renal clearance studies were performed in 1 patient.

## RESULTS

The results obtained with kanamycin in this group of patients were generally favorable. In 3 patients an excellent response was noted, 7 were classed as having a good response, 1 patient had a poor response, and 3 patients died (table I).

## CASE REPORTS

*Case 1.* L. B., a 26 year old Negro man, a heroin addict, was admitted to the hospital because of chills, fever, and cough. Examination revealed an acutely ill and toxic man. A pleural fric-

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TABLE I  
Results in 14 Patients

Pt.	Age, yr.	Race	Sex	Diagnosis	Cultures	In vitro kana- mycin sensitivities, μg./ml.	Antibiotics prior to kanamycin	Total kana- mycin, Gm.	Duration of kana- mycin therapy, days	Response	Toxicity	Comments
1 A. M.	16	N	F	Post-partum endometritis	1. Lochia, hemolytic <i>Staph. aureus</i> , coag- ulase-negative, <i>E. coli</i> , <i>A. aerogenes</i> , paracolon species 2. Urine, hemolytic <i>Staph. aureus</i> , coag- ulase-positive, <i>Escherichia</i> species, paracolon species	<i>Staph. aureus</i> , 1 <i>A. aerogenes</i> , 3 <i>E. coli</i> , 5 Paracolon, 10	Penicillin, streptomycin	26.5	13	Good	None	
2 L. B.	24	N	M	Tricuspid endocar- ditis, septicemia, septic pulmonary emboli	Blood cultures, he- molytic <i>Staph. aureus</i> , coagulase- positive (3) Blood cultures, he- molytic <i>Staph. aureus</i> , coagulase- negative (4), lochia, <i>Staphylococcus</i> type uncertain, overgrown with <i>Escherichia</i> and <i>Proteus</i> species	1	Penicillin, erythromycin	91.5	44	Excellent	Timmitus, not documented	
3 B. J. B.	22	N	F	Post-partum endometritis, septicemia	Blood cultures, he- molytic <i>Staph. aureus</i> , coagulase- negative (4), lochia, <i>Staphylococcus</i> type uncertain, overgrown with <i>Escherichia</i> and <i>Proteus</i> species	0.2-0.3	Streptomycin, penicillin, chloramphenicol, erythromycin, nitrofurantoin	27	18	Good	None	
4 J. O.	51	W	M	<i>Pseudomonas</i> lung abscess, carcinoma of tongue	Sputum, <i>Ps. aeruginosa</i> (3)	2	None	21	12	Good	None	
5 W. B.	76	N	M	<i>Proteus</i> septicemia, chronic pyelone- phritis	Throat, <i>Proteus</i> , blood, <i>Proteus</i> (2), urine, <i>Proteus</i> No pathogen isolated	10	Chloramphenicol, streptomycin	24	16	Good	None	
6 D. B.	21	N	F	Post-partum endo- metritis, possible endocarditis		—	Penicillin, streptomycin, chloramphenicol, erythromycin	21	7	Excellent	Sterile abscess at injection site	
7 C. H.	51	W	M	Staphylococcal pneumonia	Bronchial washings, hemolytic <i>Staph. albus</i> , sputum, he- molytic <i>Staph. albus</i>	Not done for kanamycin	Chloramphenicol, streptomycin, novobiocin, tracycline, isoniazid	22.5	15	Good	Acute renal tubular necrosis covered	Renal biopsy confirmed
8 C. G.	7	W	M	<i>Aerobacter</i> menin- gitis, cerebral con- cussion, epidural hematoma, multiple scalp lacerations	Spinal fluid, <i>Aero- bacter</i> , scalp wound, <i>Aerobacter</i>	Moderately sensi- tive to 10 and sensitive to 30	Chloramphenicol, penicillin, sulfisoxazole	18.5	21	Death	Proteinuria ap- peared after 10 days on kanamycin	<i>Aerobacter</i> absent from spinal fluid on two occa- sions after kanamycin began

Table I Continued on Page 597

TABLE I (Continued)  
Results in 14 Patients

Pt.	Age, yr.	Race	Sex	Diagnosis	Cultures	In vitro kana- mycin sensitivities, $\mu\text{g./ml.}$	Antibiotics prior to kanamycin	Total dose kana- mycin, Gm.	Duration of kana- mycin therapy, days	Response	Toxicity	Comments
9 D. M.	25	N	M	Staphylococcal lung abscess, Ludwig's angina	Tracheostomy, hemolytic <i>Staph. aureus</i> , coagulase-positive, bronchial washings, hemolytic <i>Staph. aureus</i> , coagulase-positive	Not done for kanamycin	Penicillin, streptomycin	34.5	23	Good	None	
10 N. J.	31	N	F	Subcutaneous abscesses, multiple; Pulmonary tuberculosis, far-advanced, active	Abscesses, hemolytic <i>Staph. aureus</i> , coagulase-positive (4), urine, hemolytic <i>Staph. aureus</i> , coagulase-positive	1	Oxytetracycline, streptomycin, chloramphenicol, sulfisoxazole	63.5	36	Good	Diffuse maculopapular eruption during second course of kanamycin	1. The larger subcutaneous abscesses were drained surgically. 2. The second course of kanamycin was given as antituberculous therapy
11 G. H.	78	W	M	Staphylococcal pneumonia	Sputum, hemolytic <i>Staph. aureus</i> , coagulase-positive	Not done for kanamycin	Penicillin	6.5	4	Death	None	
12 P. W.	21	N	F	Pelvic peritonitis after hysterectomy	Lochia, enterococcus and hemolytic <i>Staph. aureus</i> , coagulase-positive, urine, hemolytic <i>Staph. aureus</i> , coagulase-positive	Not done for kanamycin	Penicillin, streptomycin, erythromycin, chloramphenicol	10.5	7	Excellent	None	
13 E. G.	15	N	M	Staphylococcal septicemia, arthritis, subcutaneous abscess, osteomyelitis	Blood, hemolytic <i>Staph. aureus</i> , urine, abscess, coagulase-positive joints	1	None	16	10	Poor	None	1. Blood levels of kanamycin, 6.7, 10.0, 10.0 $\mu\text{g./ml.}$ at 2, 4, 6 hr., respectively, after 0.5 Gm. kanamycin intramuscularly. 2. Staphylococci susceptible to 1.0 $\mu\text{g./ml.}$ before and after 10 days of kanamycin
14 M. B.	50	N	F	Staphylococcal meningitis and septicemia, diabetes mellitus with Kimmelstiel-Wilson syndrome, arteriosclerotic heart disease, class IV D	Blood, hemolytic <i>Staph. aureus</i> , coagulase-positive (4), spinal fluid, sterile	3	Chloramphenicol, penicillin	7.5	5	Death	None	Cerebrospinal fluid findings consistent with bacterial meningitis

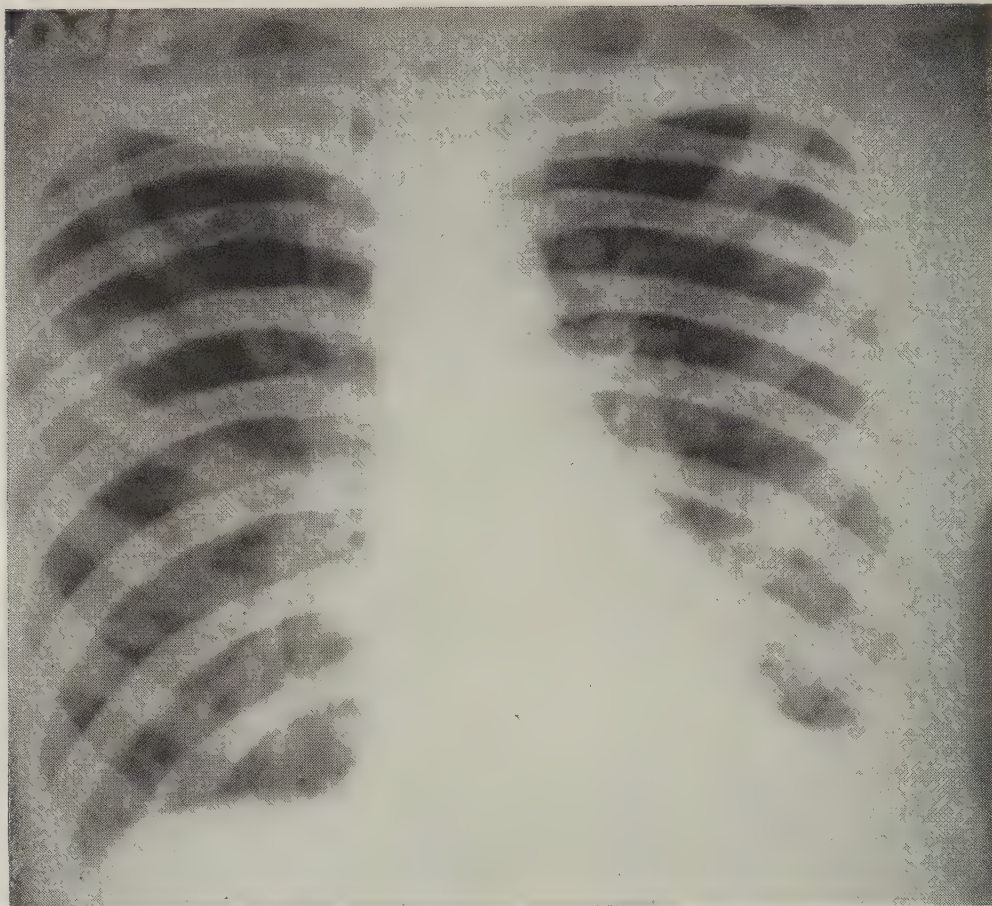


FIG. 1. Case 1. Chest roentgenogram illustrates multiple, septic pulmonary emboli present at the beginning of treatment.

tion rub was detected over the left anterolateral thoracic area, but the physical examination was otherwise not remarkable. Chest roentgenograms were consistent with multiple septic pulmonary emboli. Three blood cultures taken on admission yielded a hemolytic *Micrococcus pyogenes* var. *aureus*, coagulase positive. In vitro sensitivity studies revealed this *Staphylococcus* to be susceptible to the majority of antibiotics except penicillin. Sensitivity to kanamycin was found to be 1.0  $\mu\text{g./ml}$ .

Penicillin and erythromycin were used in combination for the first six days of hospitalization, but kanamycin replaced penicillin at this stage because of an unsatisfactory febrile response and the sensitivity studies. Erythromycin was continued with kanamycin for eight days. Kanamycin was given in a dosage of 1.5 Gm. daily for the first four days, then 3.0 Gm. daily for 17 days, and 1.5 Gm. daily for the final 23 days.

The temperature became normal one week after kanamycin was started, serial chest roentgenograms demonstrated progressive resolution of the septic pulmonary emboli, and numerous blood cultures were sterile. The patient was discharged after 54 days of hospitalization, during which time he received 91.5 Gm. of kanamycin. He complained of tinnitus when seen in the clinic six weeks after discharge but failed to cooperate when scheduled for specific evaluation of this complaint.

**Case 2.** E. G., a 15 year old Negro boy, entered the hospital with complaints of a "boil" on his back plus pain involving the right hand and both ankles. Examination revealed an acutely ill, toxic, febrile boy with a subcutaneous abscess over the left flank area and evidence of an acute arthritis of both ankles and the fourth right metacarpophalangeal joint. Smear of material aspirated from the latter joint and the abscess revealed clusters of gram-positive cocci. Nine consecutive blood cultures plus culture of material aspirated from the joints and the abscess, as

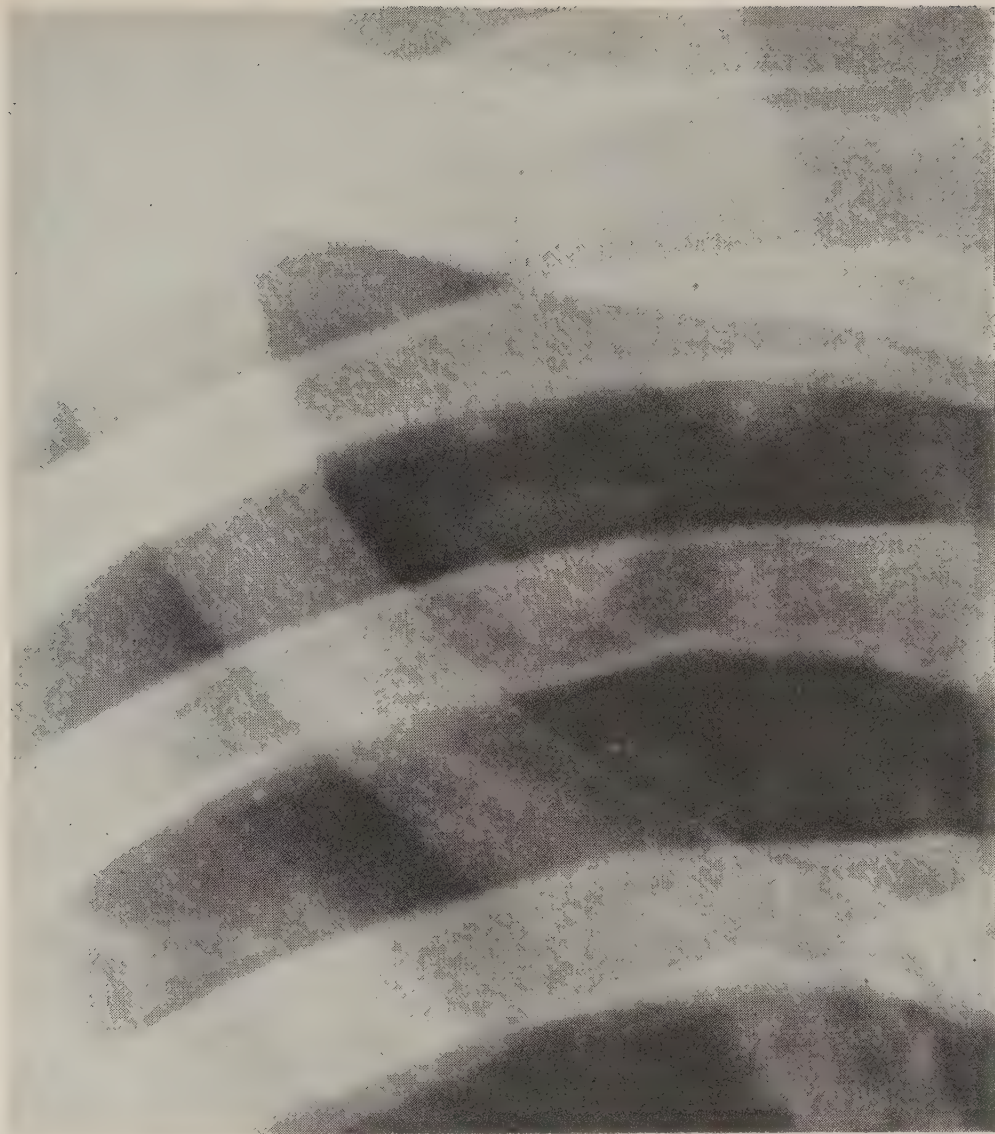


FIG. 2. Case 1. Close-up of embolic lesion in the first right anterior interspace demonstrates highlight formation.

well as urine cultures, yielded a hemolytic *M. pyogenes* var. *aureus*, coagulase positive, which was sensitive to all the commonly employed antibiotics. In vitro sensitivity to kanamycin was 1.0  $\mu\text{g./ml.}$

Initial treatment consisted of 0.5 Gm. of kanamycin every eight hours for eight days, followed by two days of 0.5 Gm. every six hours. Although the joint findings subsided somewhat and the temperature assumed a lower range, the patient did not appear to be responding well. Pain, extreme tenderness, and heat developed over both lower tibiae, there was radiographic evidence of osteomyelitis of the right tibia, and blood cultures continued to be positive for the duration of kanamycin therapy. In vitro sensitivity studies for organisms isolated after 10 days of treatment were identical to those determined initially. Blood levels of kanamycin were determined prior to discontinuing the drug and were 6.7, 10.0, and 10.0  $\mu\text{g./ml.}$  at two, four, and six hours, respectively, after 0.5 Gm. intramuscularly.

Because of the persistence of positive blood cultures, kanamycin was replaced by penicillin, the blood stream was sterilized, and gradual progressive improvement was noted.

Case 3. C. H., a 51 year old white man, was hospitalized because of a severe pneumonia.

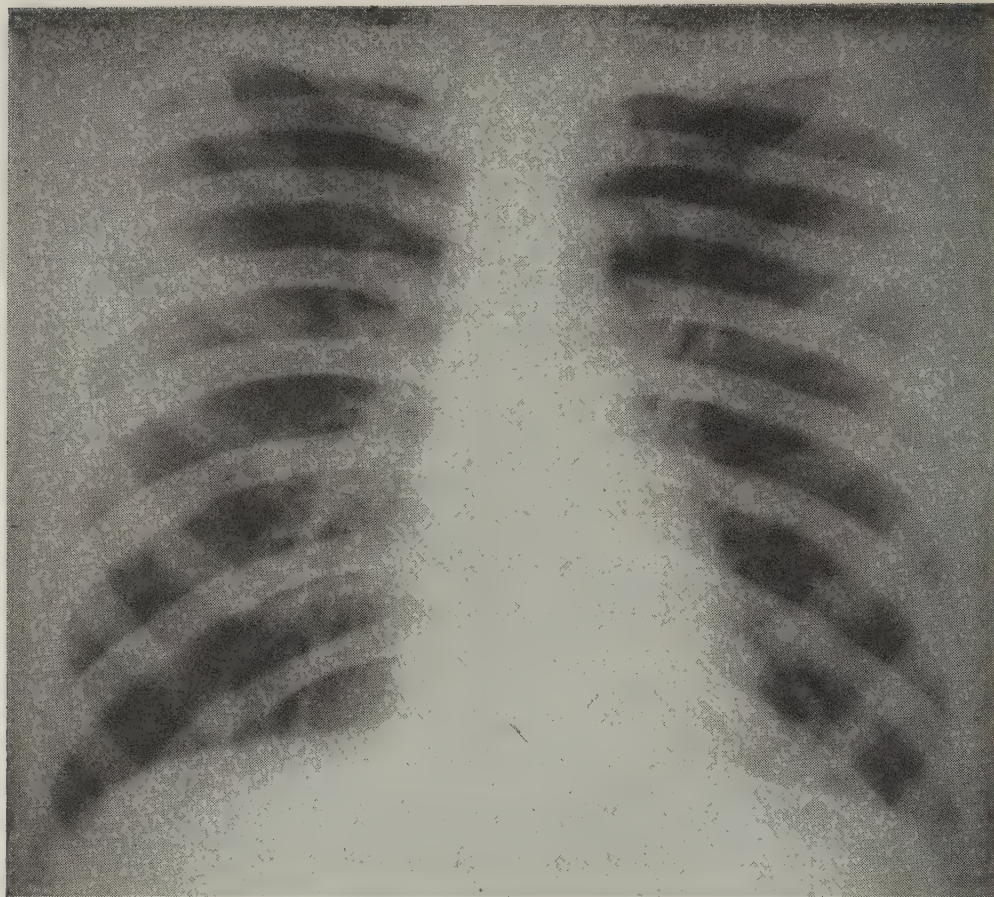


FIG. 3. Case 1. Chest roentgenogram at the completion of kanamycin therapy reveals almost complete resolution.

Past history was remarkable in respect to two previous episodes of pneumonia, removal of a renal calculus 20 years earlier, and cystoscopy for unknown reasons three years earlier.

Clinically the patient was acutely ill and toxic. Physical examination and chest roentgenograms were compatible with a right upper lobe pneumonia. A hemolytic *M. pyogenes* var. *albus* was cultured from the sputum and bronchial washings. A variety of antibiotics, including tetracycline, chloramphenicol, novobiocin, streptomycin, and isoniazid, failed to influence the course of the disease.

Kanamycin, 0.5 Gm. every eight hours, was begun and a good clinical response was noted. After two weeks of kanamycin therapy, during which time the patient had become afebrile and essentially asymptomatic, he developed fever, delirium, and oliguria. Twenty-one Gm. of kanamycin had been received up to this point. The blood urea nitrogen, which had been 31 mg./100 ml. prior to the start of therapy, varied between 16 and 18 mg./100 ml. during the two weeks of therapy. Urinalyses, before and during kanamycin, revealed intermittent proteinuria and pyuria and random specific gravities above 1.020.

On the day after the appearance of fever, delirium, and oliguria, the urine output was 15 ml., but rose gradually over the next three days to a 24 hour volume of 1650 ml. The peak blood urea nitrogen was 75 mg./100 ml., gradually declining to 30 mg./100 ml. A percutaneous renal biopsy one month after this incident revealed healing acute tubular necrosis, focal chronic pyelonephritis, and arteriolar nephrosclerosis. At the time of discharge, some residual atelectasis and shrinkage of the right upper lobe persisted.

The 3 cases classed as showing an excellent response represented, clinically, staphylococcal tricuspid endocarditis, posthysterectomy pelvic peritonitis, and postpartum endometritis. The first 2 were due to hemolytic *Staph. aureus*, while the

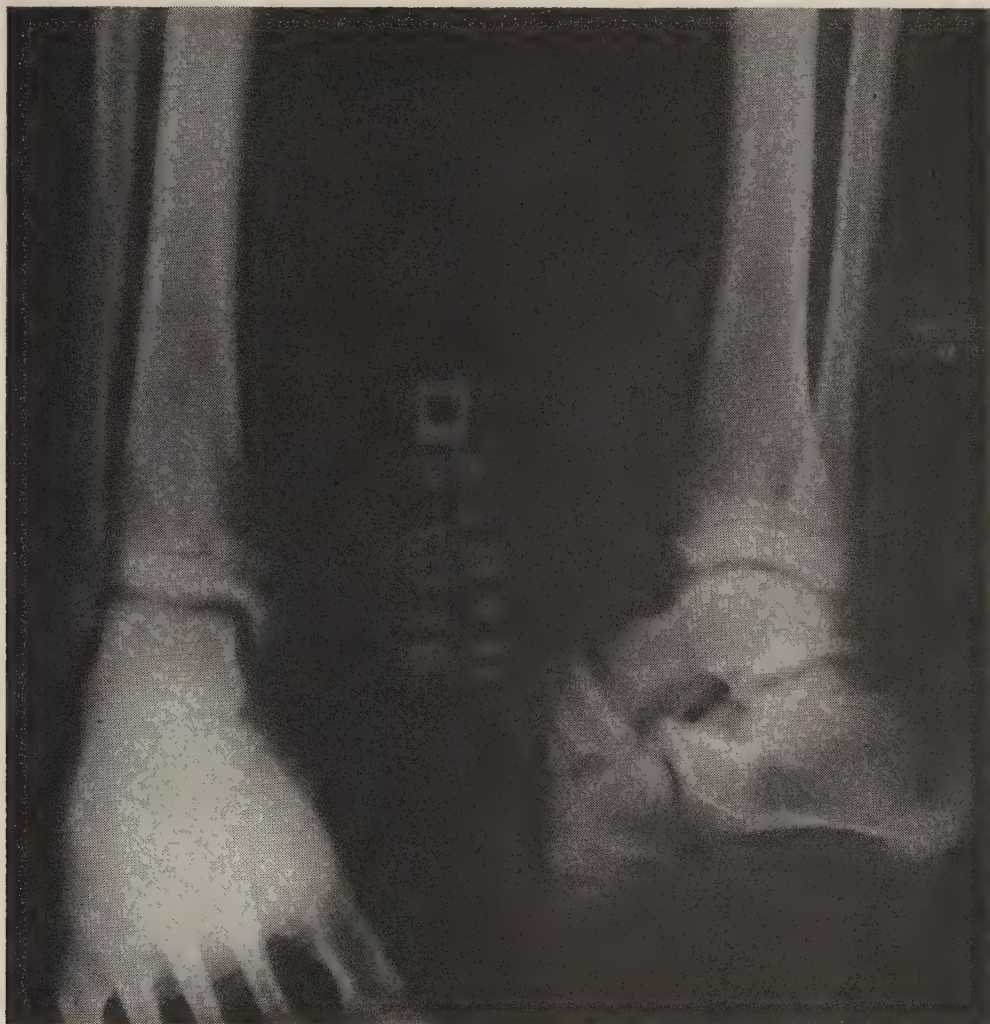


FIG. 4. Case 2. Roentgenogram of the right ankle shows osteomyelitis of distal end of the tibia. The patient was improving rapidly at this time, one week after penicillin had replaced kanamycin.

latter was not proved bacteriologically, but all 3 had responded unsatisfactorily to various combinations of antibiotics prior to kanamycin therapy.

Seven cases were graded as manifesting a good response to kanamycin therapy. These included 2 cases of post-partum endometritis, 1 of which was associated with septicemia due to hemolytic *Staph. aureus*, while the other case was a "mixed" infection. Two cases of lung abscess were also included in this category, 1 due to hemolytic *Staph. aureus* and the other due to *Pseudomonas aeruginosa*. Single cases of pneumonia due to hemolytic *Staphylococcus albus*, *Proteus* septicemia, and multiple subcutaneous abscesses due to hemolytic *Staph. aureus* completed the group of good responses. These 7 patients all had convincing, definitely favorable responses to kanamycin, and 6 of these had had poor results with previous antibacterial drugs.

The response to kanamycin was classed as poor in 1 patient who had a subcutaneous abscess, septicemia, and pyogenic arthritis due to hemolytic *Staph. aureus*. The drug failed to eliminate staphylococci from the blood or to prevent



FIG. 5. Case 2. Roentgenogram of the right ankle two weeks after the institution of penicillin therapy indicates definite resolution.

development of acute osteomyelitis. The patient subsequently had an excellent response to penicillin.

Three patients in this series died during treatment with kanamycin. Two of these patients had purulent meningitis, 1 due to *Aerobacter aerogenes* and associated with severe head trauma, and the other due to hemolytic *Staph. aureus* in a severe diabetic with Kimmelstiel-Wilson syndrome and congestive heart failure. The other death occurred in an elderly man with advanced generalized deterioration and staphylococcal pneumonia. He died after receiving 13 doses of kanamycin.

*M. pyogenes* var. *aureus* was involved in 9 of the patients in this study. In vitro sensitivity studies in 7 of these cases revealed the staphylococci to be susceptible to 0.2 to 3  $\mu\text{g.}/\text{ml.}$  of kanamycin, the majority being sensitive to 1  $\mu\text{g.}/\text{ml.}$  The *Ps. aeruginosa* implicated in case 4 was found to be sensitive to 2.0  $\mu\text{g.}$  of kanamycin per ml., and the *Proteus* isolated from the blood in case 5 was susceptible to 10  $\mu\text{g.}/\text{ml.}$  The *A. aerogenes* cultured from the spinal fluid in case 8 was moderately sensitive to 10  $\mu\text{g.}/\text{ml.}$  and sensitive to 30  $\mu\text{g.}/\text{ml.}$  In case 1, culture yielded paracolon, *Escherichia coli*, *A. aerogenes*, and *M. pyogenes* var. *aureus* sensitive to 10, 5, 3, and 1  $\mu\text{g.}/\text{ml.}$ , respectively, of kanamycin.

Serious toxic effects were noted in 1 patient and possibly in another. In the single instance of definite toxicity, acute renal tubular necrosis occurred and was documented by percutaneous renal biopsy. This case is described in detail in an illustrative case report. The other instance involves only possible toxicity, based entirely on the patient's complaint of tinnitus. This symptom occurred six weeks after completion of a 44 day course of kanamycin, during which time the patient

received 91.5 Gm. of the drug. He failed to appear for specific tests of vestibular function that had been scheduled.

Several minor complications were recorded among the patients in this series. Two patients developed sterile abscesses at injection sites, and 1 patient developed mild proteinuria after receiving kanamycin for 10 days. This latter patient was critically ill from head trauma and an associated *Aerobacter* meningitis. Another patient, who had manifested multiple drug sensitivities while receiving treatment for tuberculosis, developed a diffuse maculopapular rash during a second course of kanamycin therapy. The eruption cleared rapidly after withdrawal of the drug.

#### DISCUSSION

In discussing the results obtained with kanamycin in this study, we wish to re-emphasize that the project was purposely oriented toward a critical evaluation of the drug in severe infections. Studies organized in this fashion provide clearer end points for assessment of the ultimate practical value of the drug.

Kanamycin was generally effective and reliable in the treatment problems encountered in this investigation. Particularly impressive and encouraging were the constant laboratory susceptibility of staphylococci to kanamycin and the general effectiveness of the drug in severe staphylococcal infections. Although kanamycin was considered to have been lifesaving in several such instances, it was an equally striking and unequivocal failure in one staphylococcal infection (case 13). This failure occurred in spite of in vitro susceptibility of the organism and blood levels of kanamycin that seemed to have been more than adequate. The explanation for this unsatisfactory result falls into the realm of speculation, but such paradoxes in staphylococcal infections are well known.<sup>1</sup> In sum, however, our experience with kanamycin in severe staphylococcal infections suggests that it is a valuable new weapon in this area of therapeutic difficulty.

In the sphere of gram-negative bacillary infections, our experience was quite limited, although such infections seemed to be generally well controlled by kanamycin. We noted with interest that the single case involving *Ps. aeruginosa* responded well to treatment and that the organism was sensitive to 2  $\mu\text{g./ml.}$  of kanamycin.

The 3 deaths in this series were not a result of drug failure in our opinion. In both cases of meningitis there were serious associated problems, and survival in either instance would have been truly spectacular. One must be aware of the fact that the mortality in any type of bacterial meningitis continues to be quite high in spite of exceedingly potent antibacterial agents, such as penicillin for pneumococcal meningitis. *Aerobacter* was eliminated from the spinal fluid in the case of meningitis due to this organism, and the spinal fluid cell count diminished from more than 900 to less than 200/cu. mm. after five days of kanamycin therapy in the case of meningitis due to hemolytic *Staph. aureus*. The other death occurred in an elderly, debilitated man with staphylococcal pneumonia. He received only 13 doses of kanamycin, and this, plus his poor general status, would suggest that factors other than drug failure were related to his death.

Although major toxicity was well documented in 1 patient and suggested in another, our experience in a reasonably large number of patients, including many not discussed in this report, would tend to support the impression that kanamycin is not associated with an inordinate incidence of toxicity in the manner in which the drug is used for acute bacterial infections.

Kanamycin was used in a variety of severe infections, the majority due to *M. pyogenes* var. *aureus*, with generally good results. One definite drug failure was encountered in the staphylococcic group, and a suitable explanation was not apparent. A somewhat smaller number of gram-negative infections responded well to kanamycin, including 1 case of *Ps. aeruginosa* lung abscess. Acute renal tubular necrosis occurred in 1 patient, but no other definite serious toxicity was recorded.

As a result of our experience with kanamycin in this small group of patients, we believe that it is an important and valuable addition in the field of antibiotics but do not advocate its indiscriminate use in routine infections. Use of this drug should be limited to selected infections, particularly those due to staphylococci, *Proteus*, and *Pseudomonas* organisms, for in the area of organisms resistant to the presently available antibiotics, a new and effective agent, such as kanamycin, is urgently needed.

## BIBLIOGRAPHY

1. FINLAND, M., AND JONES, W. F., JR.: Staphylococcal infections currently encountered in a large municipal hospital: some problems in evaluating antimicrobial therapy in such infections, *Annals New York Acad. Sc.* 65:191-205, Aug. 31, 1956.

# Evaluation of Kanamycin in the Local Treatment of Pyodermias

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While many effective topical agents for the pyodermias are in common use,<sup>1</sup> it is necessary to continue the search for new ones. Side reactions to any drug may develop, precluding its further use; resistance is always a factor to be considered; and efforts to discover more effective agents are always in order.

Previous to this study, neomycin ointment<sup>2,3</sup> was our choice of topical remedy. We have found the drug rapidly effective in practically all cases, and contact dermatitis from its use was not common, although of late we have noted several instances of side reactions in ointments containing neomycin and a steroid.

This study is essentially a comparison of the results obtained with neomycin over a period of years with kanamycin. In several cases photographs before and during treatment were made.

In our study, kanamycin ointment and cream, each containing 5 mg./Gm. of kanamycin base activity as the sulfate, were used in the treatment of cases of pyodermias for a three month period. Our series included 20 patients with impetigo, 2 with infectious eczematoid dermatitis, 2 with sycosis vulgaris, and 1 with folliculitis barbae. In all cases, cultural studies with sensitivity tests were made. The hemolytic *Micrococcus* was the most common offender; at times, the *Streptococcus* was cultured as a mixed infection. The organisms were sensitive in vitro to kanamycin and most other antibiotics, except penicillin and triple sulfonamides. All cases were treated similarly. The patient or parent was instructed to apply the ointment or cream and spread with slight friction, using a wooden spatula. Frequency of application was every two or three hours during the waking hours.

The results were excellent in all cases of impetigo, distinct improvement being noted in 72 hours in all patients and cures in one week. Two cases of infectious eczematoid dermatitis of several months' duration responded exceedingly well. There was a failure with 1 case of sycosis, which had resisted all types of antibiotic therapy over a period of years. No instances of contact dermatitis developed in our series.

## CASE REPORTS

A. L., 57 years old, developed an eruption in 1955 on the left leg, at the site of a pigmented scar from a gasoline burn in 1943. The diagnosis was infectious eczematoid dermatitis. Kanamycin ointment was applied several times daily. Distinct improvement was noted in one week, and the infectious element was considered cured in three weeks.

A 2 year old child had a pyoderma of the toes that could be classified as dermatitis repens that had been present for several months. The culture showed *Streptococcus hemolyticus* and hemolytic *Micrococcus aureus*. There were an associated lymphangitis and lymphadenopathy. On combined treatment with erythromycin given orally and kanamycin ointment given topically, there was distinct improvement in one week and a cure in three weeks.

## BIBLIOGRAPHY

1. MILLER, J. L.; SLATKIN, M. H., AND JOHNSON, B. A.: Evaluation of bacitracin in local treatment of pyogenic infections, *Arch. Dermat. & Syph.* 60:106-120, July, 1949.
2. LIVINGOOD, C. S.; HEAD, C. E.; SUTTER, R. M., AND ENGLE, F. B., JR.: Comparative effects of neomycin and other antibiotics on pyogenic bacteria, *A.M.A. Arch. Dermat. & Syph.* 69:43-57, Jan., 1954.
3. KILE, R. L.; WELSH, A. L., AND MCAFEE, G. D.: Topical use of neomycin (preliminary report), *Arch. Dermat. & Syph.* 62:911, Dec., 1950.

# Clinical Evaluation of Kanamycin

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Kanamycin\* is a new antibiotic, isolated by Umezawa et al<sup>9</sup> from *Streptomyces kanamyceticus*. It is a basic, water soluble, and stable drug.<sup>1</sup> Chemically, it is related to neomycin and to streptomycin.

In vitro, kanamycin exhibits good bactericidal activity against a wide variety of gram-negative and gram-positive organisms.<sup>5,6</sup> Studies to date suggest that development of resistant strains will not be a major problem with this drug.

Animal studies have revealed nephrotoxicity and eighth nerve toxicity; these changes were less severe than those noted with neomycin, streptomycin, and dihydrostreptomycin in similar dosage.<sup>2</sup> Two closely related forms of the drug—kanamycin A and B—are produced. Kanamycin B is more toxic to animals than kanamycin A.<sup>7</sup>

A previous study from this group<sup>3</sup> presented findings in a group of 81 patients treated parenterally and 7 treated orally with kanamycin. The present report covers these same patients, with 25 additional patients treated parenterally and 3 orally; this report includes a longer follow-up on the first group for further evaluation of toxicity and therapeutic effectiveness. Among the 81 original cases are 20 patients studied by another group from these hospitals; their report concerns primarily the nephrotoxicity of kanamycin.<sup>10</sup>

## MATERIALS AND METHODS

The patients were all hospitalized. Nine of these patients were female and 97 male. The age range was from 12 to 87 years. The types of infections treated are noted in tables I, II, and IV. Patients treated for less than one day are not included, unless toxicity was encountered.

The lots of kanamycin used parenterally were 57K393, 58K34, and 58K89, and orally, 57A545 and 58J1500. Lot 57K393 contained 95 per cent kanamycin A and 5 per cent B; the other parenteral lots contained 97 to 98 per cent kanamycin A.

Nine patients received the drug intravenously for up to four days with a maximum dose of 3 Gm./day. It was usually dissolved in 5 per cent dextrose in water at a concentration of 1 Gm./l. and administered slowly over a period of 8 to 10 hours.

The intramuscular preparation of kanamycin was supplied in vials containing either 500 mg. of kanamycin base as the sulfate in 2 ml. of an aqueous solution or 1 Gm. in 3 ml. Generally, 0.5 to 1.0 ml. of 1 to 2 per cent procaine or  $\alpha$ -diethyl-amino-2,6-aceto-xylylidide was added to the vial prior to withdrawal for injection. Intramuscular injections were made deep into the gluteal muscles, generally every 8 to 12 hours.

The oral preparation was supplied both in capsules and as syrup. The capsules

This study was supported, in part, by a grant in aid from Bristol Laboratories Inc.

\* The trade name of Bristol Laboratories Inc. for kanamycin is Kantrex.

TABLE I  
*Clinical Results with Parenteral Kanamycin*

Type of infection	Results					
	Unable to evaluate	Good	Satis.	Poor	Good, but relapse on therapy	Good, but relapse after therapy
<i>Staphylococcal Infections</i>						
Pneumonia	2	12			1	1
Endocarditis		1			1	1
Septicemia		3		1		
Mycotic aneurysm		1				
Empyema	1	1				
Osteomyelitis		1	2	4		
Soft-tissue infections	1	7	2	4		
Miscellaneous						
Peritonitis	1					
Bursitis, purulent		2				
Chronic otitis media		1				
Bronchiectasis			1			
Intra-abdominal abscesses, fistulae		2				
Omphalitis		1				
Total	5	32	5	9	2	2
<i>Pneumococcal Infections</i>						
Pneumonia		3		1		
Endocarditis				1		
Total		3		2		
<i>Miscellaneous Infections</i>						
Subacute bacterial endocarditis ( <i>Pseudomonas</i> )				1*		
Bacteremia						
<i>Pseudomonas</i>				1†		
<i>Aerobacter aerogenes</i>		1				
<i>Escherichia coli</i>		3		1		
Intermediate paracolon		1				
<i>Bacteroides</i> infections						
Septicemia, empyema				1		
Pelvic abscess				1		
Brucellosis ( <i>Brucella suis</i> )				1		
Paratyphoid B		1				
Atypical acid-fast bacilli disease (pulmonary)		1				
Pneumonitis ( <i>Pseudomonas</i> )				1		
Peritonitis, abdominal wound infection (mixed)		1				
Chronic intra-abdominal abscess, multiple fistulae						
<i>Proteus</i>				1		
Mixed				1		
Draining abdominal wall sinus ( <i>Pseudomonas</i> , <i>E. coli</i> )				1		
Total		8		10		
<i>Infections of Uncertain Etiology</i>						
Pneumonia	1	4		1		
Prostatitis, seminal vesiculitis		1				
Empyema		1				
Total	1	6		1		

\* Concomitant therapy with three other drugs.

† Concomitant therapy with four other drugs.

TABLE II  
Clinical Results with Parenteral Kanamycin in *Pyelonephritis*

Patient no.	Organism	Days treated	Daily dose, mg./Kg.	Cultures during therapy	Cultures at end of therapy	Bacteriological follow-up	
						No. weeks post-treatment	Later cultures
13	<i>Pseudomonas</i> , $>10^3$ /ml.	17*	27.5	Negative	Negative	9	Negative
24	<i>P. morgani</i> ; <i>P. mirabilis</i> , $>10^3$ /ml.	19	18	Negative	Negative	16	Negative $\times 2$
31	<i>P. mirabilis</i> , $>10^3$ /ml.	28	48	Negative	<i>Pseudomonas</i> , $<10^3$ /ml.	1	<i>P. mirabilis</i> , $<10^3$ /ml.
35	<i>E. coli</i> , $>10^5$ /ml.	22	Subsequent therapy with another agent		Negative	4	<i>P. mirabilis</i> , $<10^3$ /ml.; <i>Pseudomonas</i> , $<10^3$ /ml.
36	<i>P. mirabilis</i> , $>10^3$ /ml.	6				1	Negative
						1	Negative
37	Coliform bacteria	14	25	Negative $\times 3$	Negative	6	Negative
38	<i>Pseudomonas</i> , $>10^3$ /ml.	6	50		Negative		Negative
			Subsequent therapy with another agent (prophylactically?)		Negative	12	Negative
						1	Negative
40	<i>E. intermedium</i> <i>Pseudomonas</i>	19	27	<i>Pseudomonas</i> (light growth)	<i>Candida</i>		<i>Pseudomonas</i> <i>Candida</i> <i>P. rettgeri</i> , $10^5$ ; <i>A. aerogenes</i> , $10^3$ ; $\beta$ -hemolytic enterococci
43		31	25	<i>Pseudomonas</i> $\times 2$ <i>Candida</i>		1	
45	<i>Proteus</i>	27	48	Negative $\times 2$	Negative	7	Negative
61	<i>Proteus</i> , $>10^3$	15*	13	Negative $\times 2$		14	Negative
						2	Negative
						4	Negative

Table II Continued on Page 609

TABLE II (Continued)  
Clinical Results with Parenteral Kanamycin in Pylonephritis

Patient no.	Organism	Days treated	Daily dose, mg./Kg.	Cultures during therapy	Bacteriological follow-up		
					Cultures at end of therapy	No. weeks post-treatment	Later cultures
66	<i>E. intermedium</i>	9	30		Negative	8	<i>E. intermedium</i> ; <i>Proteus</i>
67	<i>E. coli</i>	8	30		Negative	2	Negative
						7	Negative
69	<i>E. coli</i> , $>10^3$ /ml.; Enterococcus, $>10^3$ /ml.; <i>P. mirabilis</i> , $>10^3$ /ml.	9	15			8	Negative
						1	<i>Str. viridans</i> , $>10^3$ /ml.;
						5	Negative,
						10	<i>P. mirabilis</i> , $>10^5$ /ml.
						(Lost to follow-up)	<i>E. coli</i> , $>10^5$ /ml.
							Enterococcus, $>10^5$ /ml.
75	Coliform bacteria	16	19	<i>Bacteroides</i>			
76	<i>Pseudomonas</i>	5	27		<i>Pseudomonas</i> , $>10^5$		
77	<i>Proteus</i>	12	20		<i>Bacteroides</i>	6	<i>Proteus</i> , $>10^3$
79	<i>P. morganii</i> , <i>E. coli</i>	10	7	<i>Candida</i> $\times 2^\dagger$	<i>Pseudomonas</i> ; <i>Candida</i>		
87	Intermediate paracolon	17	9		<i>Pseudomonas</i> , $10^4$ ; Enterococcus, $>10^4$		
99	<i>E. coli</i>	10	13.2	<i>Pseudomonas</i>			
101	<i>A. aerogenes</i>	13	21	Negative		2	Negative
104	<i>E. coli</i> , $>10^5$ ; <i>Achromobacter</i> , $>10^5$ ; <i>Str. faecalis</i>	8	16		<i>Str. faecalis</i> , $10^4$		

\* Given in two courses of therapy.

† Also present before treatment.

each contained 500 mg. of kanamycin base activity; the syrup contained 500 mg. of kanamycin base activity as the sulfate per 5 ml. Kanamycin was used orally in a total dosage of 6 Gm./day (of base activity), except in 1 smaller patient who received 4 Gm./day; the drug was given every six hours. The 8 patients treated orally for purposes of intestinal sterilization received, in addition, four loading doses of 1 Gm. each at hourly intervals on the first day. (Castor oil, 60 ml., was given with the first of these doses in order to hasten the passage of the drug through the gastrointestinal tract.) These patients all received a standard hospital diet. The dose of kanamycin in all cases was between 100 and 130 mg./Kg. body weight/day. The *Salmonella* infections were treated for one week and the others for 3 to 25 days.

Sensitivity tests were carried out by a tube dilution technique employing heart infusion broth (Difco), Sorenson's pH 8.0 phosphate buffer as the diluent for the antibiotic, and an inoculum consisting of a 1:10,000 dilution of an 18 hour broth culture.<sup>6</sup> Serum assays were done by Bristol Laboratories.

The earlier patients were studied serially with a battery of tests prior to, during, and after kanamycin therapy, in order to evaluate toxicity carefully;<sup>3</sup> later studies were restricted to complete blood counts, urinalyses, serum creatinines, pure tone audiograms, and caloric stimulation tests. The patients were questioned regularly, particularly with regard to tinnitus and symptoms suggestive of vestibular disturbance.

## RESULTS

There was no particular difference noted between the three parenteral lots of drug and between the two oral preparations (one capsules and one syrup) as to either effectiveness or toxicity.

*Tolerance.* Kanamycin was tolerated well intravenously without evidence of toxicity. One patient developed a mild thrombophlebitis but was concurrently getting 50 million units of penicillin intravenously daily.

There was a high incidence of pain at the injection site with the larger doses. Usually this could be prevented or modified by adding procaine or  $\alpha$ -diethylamino-2,6-aceto-xylylides to the kanamycin prior to injection or by injecting these local anesthetics intramuscularly prior to the kanamycin injection in the same site. Despite these measures, 14 patients complained of moderate to severe local pain; this generally lasted only a few minutes and never necessitated discontinuance of treatment. One of these 14 patients had tender nodules at the injection site.

Oral kanamycin was tolerated without any nausea, vomiting, abdominal distress, or diarrhea, with the exception of patient C. U., discussed in the following; patients generally only had one or two semiformal or formed stools daily while on the drug.

C. U., a 78 year old white man with marked emphysema, received 100 mg./Kg. body weight of kanamycin in capsule form (4.0 Gm.) daily for four days, plus an additional 4.0 Gm. as a loading dose (1.0 Gm. every hour for four hours) the first day. For the first two days of therapy, this patient had some nausea, two to three watery to mucoid stools per day, and mild to moderate lower abdominal cramps and distress. These symptoms subsided considerably during the last two days of therapy. Twenty-four hours after the drug was discontinued, the patient had marked respiratory difficulty and gradually became hypotensive. He was felt to have respiratory acidosis and was placed in a Drinker respirator and given methoxamine hydrochloride (and later norepinephrine) and supportive care. Despite these measures he was in shock on and off for 24 hours and then died. Unfortunately, a stool culture was not obtained at this time; however, a quantitative stool culture on the day the drug was discontinued revealed no aerobic bacteria, small numbers of *Candida* (not *Candida albicans*), and the usual anaerobes present in average

TABLE III

Summary of Results in Treatment of Pyelonephritis with Kanamycin

Response	No.	Organism
Failure to eradicate organism during therapy	1	<i>Pseudomonas</i>
Partial eradication of organisms during therapy	1	One of three original organisms persisted ( <i>Str. faecalis</i> )
Organism eradicated and replaced with new organism(s) during therapy	7	3, <i>Pseudomonas</i> ; 1, <i>Candida</i> ; 2, <i>Bacteroides</i> ; 1, <i>Pseudomonas</i> and enterococcus
Bacteriological Response With Relapse Later		
Within one week of discontinuing drug	1	<i>Proteus</i>
Time of relapse uncertain, but less than four weeks	1	<i>Proteus</i>
Time of relapse uncertain, but less than eight weeks	1	<i>E. intermedium</i>
Between five and ten weeks after discontinuing drug	1	Three organisms
Subtotal	13	
Good Bacteriological Response		
(With no follow-up)	1	<i>A. aerogenes</i> )
With two weeks or less follow-up	3	1, <i>E. coli</i> ; 1, <i>Pseudomonas</i> ; 1, <i>A. aerogenes</i>
With four to five weeks follow-up	1	<i>Proteus</i>
With six to eight weeks follow-up	2	1, <i>Proteus</i> ; 1, <i>E. coli</i>
With 12 weeks follow-up	1	Coliform bacteria
With 14 weeks follow-up	1	<i>Proteus</i>
With 16 weeks follow-up	1	<i>Pseudomonas</i>
Subtotal	9	
Total	22	

numbers. At autopsy, focal reddening of segments of small bowel was noted; this was prominent in the distal 30 cm. of small bowel, where the mucosa was noted to be grossly altered and discolored by a dirty gray membrane. Unfortunately, a culture was not taken. The microscopic sections of the ileum revealed inflammatory changes through the mucosa and, to a lesser extent, the submucosa. Patchy areas of mucosal necrosis were encountered; here, large numbers of polymorphonuclear leukocytes and cocci resembling staphylococci were encountered.

Clinical results are summarized in tables I (all infections other than pyelonephritis treated parenterally), II and III (results in pyelonephritis), and IV (results of oral therapy).

The results in staphylococcal infections were generally excellent, considering that many of these patients were seriously ill with severe infections. The 2 patients who relapsed following treatment both represented instances in which therapy had to be stopped sooner than planned because of toxicity. There was no obvious explanation for the failure to respond in some of the staphylococcal (and other) infections; several of these patients had organisms that were sensitive both before and after treatment.

TABLE IV

Response to Oral Kanamycin, Therapy (Approximately 100 mg./Kg./Day)

Number of patients	Reason for treatment	Results	Toxicity
8	Intestinal sterilization	Excellent	? 1
2	Salmonellosis	Satisfactory	pseudomembranous ileitis

K. R. is a 41 year old white man with rheumatic heart disease with mitral stenosis, atrial fibrillation, and congestive failure, who had a mitral commissurotomy in 1953. He was admitted in February, 1958, because of increasing shortness of breath. In March, 1958, he underwent a second mitral commissurotomy because of re-stenosis of the mitral valve. Postoperatively, he developed a staphylococcal wound infection. This was treated with tetracycline and then with chloramphenicol and erythromycin. The local wound was improving when the patient became febrile again, showed evidences of peripheral embolization, developed changes in the quality of his murmur, and had blood cultures positive for a coagulase-negative *Staphylococcus aureus* (kanamycin sensitivity less than 0.45  $\mu\text{g.}/\text{ml.}$ ). Initial therapy for his bacterial endocarditis consisted of vancomycin (begun April 1, 1958); there appeared to be some response, but blood cultures remained positive, so penicillin was added. After he developed a rash, the penicillin was discontinued and replaced with bacitracin on April 7. Blood cultures remained positive while he was on vancomycin and bacitracin, so, on April 11, bacitracin was stopped and kanamycin (3.0 Gm. daily) was substituted. While on vancomycin plus kanamycin he improved, and his blood cultures became negative; however, he was still febrile. On April 22, the vancomycin was discontinued, and the patient promptly became afebrile and felt much better generally. However, serum from this patient while he was on kanamycin alone failed to show any greater bacteriostatic or bactericidal activity versus his own infecting organism than serum drawn while he was on both kanamycin and vancomycin. The possibility of drug fever due to vancomycin exists. Kanamycin alone was continued at the same dosage until April 30, when the dose was cut in half because of a rising serum creatinine (2.0 mg. per cent). On May 5 the patient developed tinnitus, and kanamycin was discontinued. Serial audiograms revealed an average perceptive decibel loss of 25 to 30 at 2000, 4000, and 8000 cycles on the right and at 4000 and 8000 cycles on the left. His conversational hearing loss is not pronounced. Follow-up has shown no clinical or bacteriological evidence of relapse to date (four month follow-up so far). Obviously, it is not possible to state whether the ototoxicity was related to the kanamycin, the vancomycin, or to both.

There were only a few pneumococcal infections treated, but the results were clearly inferior to those obtainable with several other available agents.

The patient with brucellosis (E. B., presented in the following) was a clinical and bacteriological treatment failure, despite marked sensitivity of the organism in vitro and a very adequate trial of therapy.

E. B., a 37 year old Negro slaughterhouse worker, entered the hospital with a history of fever, chills, night sweats, weakness, anorexia, headaches, and weight loss of 12 lb. during a three to four week period. Physical examination was unremarkable. The *Brucella* agglutination titer was 2 plus in a 1:1280 dilution. Several blood cultures showed *Brucella suis*. The patient became afebrile while diagnostic studies were under way but he became febrile again later, and kanamycin was started in a dose of 1 Gm. every eight hours given intramuscularly. Despite the fact that the organism was sensitive to less than 0.45  $\mu\text{g.}/\text{ml.}$  kanamycin, the fever continued and blood cultures remained positive for *Br. suis*. After three weeks of kanamycin, tetracycline was added; the fever subsided in three days and blood cultures were negative. The patient completed an uneventful recovery and was discharged.

The atypical acid-fast infection responded very well to kanamycin, but a co-existing pneumococcal infection was a clinical and bacteriological treatment failure (patient G. S.); therapy had to be changed after almost three months because of toxicity.

G. S. is a 47 year old white cook who developed fever, chills, cough productive of yellow sputum, and diarrhea, one month prior to admission. These symptoms abated, but he continued to have malaise and occasional night sweats and lost 20 lb. in weight. Four days prior to admission, he again developed his original symptoms plus pleuritic pain in the left chest. He entered the hospital at that time, and a roentgenogram revealed an infiltrate in the left upper lobe. The routine sputum culture yielded pneumococci, and the patient's initial response to penicillin was good. He soon relapsed, however, and repeat roentgenogram showed suggestions of cavitation and a small pocket of fluid. Smears of both the sputum and the empyema fluid revealed acid-fast bacilli. (Two gastric cultures of three taken at this same time subsequently showed an atypical, acid-fast bacillus; this organism was a scotochromogen and was neutral-red positive.)

At this point, the patient was switched from penicillin to kanamycin (1 Gm. daily). Initially, he did very well, but after three days he again relapsed, and pneumococci were again recovered from the sputum. With the addition of penicillin he again became afebrile; the penicillin was discontinued after one week and the kanamycin continued for a total of just under three months, when it was stopped because of tinnitus. The patient remained afebrile, gained 20 lb., and showed considerable resolution of the pulmonary infiltrate during this period. He has subsequently received isoniazid and *p*-aminosalicylic acid and continues to do well. Serial audiograms were made, and a 40 decibel perceptible loss at 2000 cycles in the left ear appeared at the time of onset of tinnitus; this has persisted. The patient has not had great difficulty with conversational hearing.

The case of paratyphoid B infection (established serologically only) responded very rapidly. Excellent results were obtained in 5 cases of bacteremia (3 *Escherichia coli*, 1 *Aerobacter aerogenes*, and 1 intermediate paracolon); the fifth case (M. F.) is discussed in the following. The 1 *E. coli* bacteremia treatment failure was a patient with acute lymphoblastic leukemia being treated with steroids, who had previously had (10 days earlier) an *E. coli* bacteremia that responded well to kanamycin; unfortunately, the 2 strains were not obtained for kanamycin sensitivity studies.

M. F. is a 47 year old white typist who was admitted for repair of a vesicovaginal fistula. Prior to surgery she was placed on steroids to facilitate cleavage of fascial planes at surgery; she also received prophylactic antibiotics. Postoperatively, the patient developed fever; despite treatment with penicillin, chloramphenicol, and nitrofurantoin, successively, her fever climbed to more than 105 F., and bacteremic shock ensued. Steroids were continued, norepinephrine was administered by continuous intravenous drip, the other antibiotics were discontinued, and kanamycin substituted. Initially, she was given 1 Gm. of kanamycin daily by slow intravenous drip. The response was dramatic; she became afebrile in 24 hours, and it was possible to discontinue the norepinephrine at this time. After three days, the kanamycin was given intramuscularly with dosage cut to 0.5 Gm./day (7.5 mg./Kg. body weight) because her serum creatinine was 4.8 (related to the shock). The creatinine fell as kanamycin was continued and reached 1.3 mg./100 ml. after an additional six days. The patient received kanamycin for a total of 17 days without any evidence of toxicity. Blood and urine cultures taken just prior to instituting kanamycin therapy yielded an intermediate paracolon. This organism, by disc sensitivity test, was resistant to tetracycline, chloramphenicol, polymyxin, and nitrofurantoin, was moderately sensitive to neomycin, and was sensitive to kanamycin. Using a tube dilution technique, this organism was completely inhibited by 7.8  $\mu$ g./ml. of kanamycin and 31.25  $\mu$ g./ml. of neomycin and was killed by 7.8 and 62.5  $\mu$ g./ml. of kanamycin and neomycin, respectively. Blood cultures remained sterile after kanamycin was started; the intermediate paracolon disappeared from the urine and was replaced by *Pseudomonas* and enterococci. Follow-up studies revealed an obstruction at the left ureterovesical junction, with hydroureter and some hydronephrosis. A second operation has just been performed; this revealed a suture obstructing the left ureter. Following removal of this suture, the patient appears to be doing very well; further studies are in progress.

Three *Pseudomonas* infections (one subacute bacterial endocarditis, one bacteremia, and a pneumonitis) were all treatment failures. The first 2 patients had previously been treated, respectively, with three and four other antibiotics, which were maintained along with kanamycin. The pneumonitis responded well later to polymyxin. The 2 *Bacteroides* infections failed to respond either clinically or bacteriologically to kanamycin.

The results in pyelonephritis (tables II and III) were generally favorable. It is of interest to note that the case in which the organism was not eradicated was a *Pseudomonas* infection; that *Streptococcus faecalis* persisted in the case with only partial eradication of organisms; and that, in those instances in which an original organism was eradicated and replaced with a new organism, the super-infecting organisms were *Pseudomonas*, *Bacteroides*, *Candida*, and enterococci. These organisms are resistant (often highly resistant) to kanamycin as a rule.

TABLE V  
Toxicity and Tolerance in 106 Patients Given Kanamycin Parenterally

Toxic reaction	No. of patients
Eighth nerve toxicity	
Audiographic changes only	13
(no subjective loss)	
Conversational loss (persistent)	4
Tinnitus and/or vertigo only	5
	22
Questionable	5
Renal toxicity	
Elevation of serum creatinine	
Definite	12
Questionable	7
Changes urine sediment	47
Hypersensitivity	
Rash	2*
Eosinophilia	9
Anaphylactic reaction	1(?)†
Drug fever	1
Hydrops of labyrinth	1
Neurotoxicity	
Odd visual complaints	2
Paresthesias	1
Peroneal palsy	1(?)
Diarrhea	1
Moderately severe to severe local pain	14

\* One patient had positive immediate skin test.

† Patient had positive immediate skin test.

Over-all appraisal of our results with parenteral kanamycin therapy suggests that, in general, results were as good with doses of 15 mg./Kg. body weight/day as with considerably larger doses. Large numbers of cases that are equivalent in all respects would have to be treated with large and small doses to shed further light on this point.

In eight instances in 7 patients, it was possible to recover bacteria of the same species as the original infecting organism after kanamycin therapy had been completed, because of inadequate response or relapse following treatment. There was no significant difference in the in vitro sensitivity to kanamycin or to neomycin between the pre- and post-treatment cultures in any of these cases.

The results of intestinal sterilization with kanamycin capsules in 5 patients and of oral treatment of *Salmonella typhimurium* infections in 2 patients have been discussed previously.<sup>3,6</sup> All aerobic bacteria were rapidly eliminated in the 5 patients, with little or no change in the anaerobic flora and relatively small increases in yeasts. The 2 *Salmonella* cases responded well, with negative cultures during treatment and at weekly intervals for three weeks post-treatment.

Three additional patients have received kanamycin orally (this time as the syrup) for purposes of intestinal sterilization. At the end of 36 hours of therapy, stool cultures revealed no aerobic bacteria, small numbers of yeasts ( $10^3$ /Gm. wet feces) in 2 cases, and the usual numbers of anaerobes (variously, *Bacteroides*, *Lactobacillaceae*, *Clostridium*, and *Fusiformis*). Table IV is a summary of results with kanamycin given orally.

#### TOXICITY

An over-all survey of toxicity appears in table V, and further details appear in tables VI through XIV.

TABLE VI  
*Toxicity Tabulation—Kanamycin Administered Parenterally*

Dose, mg./Kg./day	Total no. patients	No important toxicity	Auditory toxicity	Vestibular toxicity	Elevation of creatinine	Minor urinary sediment abnormalities
Treated Seven Days or Less						
<10	2	2				
11-15	5	4	1(?)		1(?)	2
16-20	5	5				2
21-25	2	1			1	
26-30	3	3				1
31-40	5	5				1
41-50	3	1	2			
Total	25	21	2 and 1(?)		1 and 1(?)	6
Treated 8 to 14 Days						
<10	1	1				
11-15	6	6				1
16-20	6	5	1		1(?)	1
21-25	7	6	1		1	2
26-30	6	3	2 and 1(?)			2
31-40	2	2				
41-50	2	2				2
Total	30	25	4 and 1(?)		1 and 1(?)	8
Treated 15 to 21 Days						
<10	1	1				
11-15	4	1	2	1	1	2
16-20	4	4				2
21-25	8	4			4	5
26-30	3	3				1
31-40	3		1 and 1(?) *	1(?) *	1 and 2(?) †	3
41-50	7		7	2	2 and 1(?)	7
51-60	2	2				1
Total	32	15	10 and 1(?)	3 and 1(?)	8 and 3(?)	21
Treated 22 to 28 Days						
11-15	1	1				1
16-20	1	1				1
21-25	2	1	1			1
26-30						
31-40						
41-50	2		2	1	1	1
51-60	3	1	1 and 1(?) *			2 and 1(?) *
Total	9	4	4 and 1(?)	1	1	6 and 1(?)
Treated 29 to 56 Days						
11-15	1		1			
16-20	1	1				1
21-25	2				1 and 1(?)	1
26-30	1	1				1
31-40	1	1				
41-50						
51-60	1	1				1
>60	1		1(?) *	1(?) *	1(?) *	1(?) *
Total	8	4	1 and 1(?)	1(?)	1 and 2(?)	4 and 1(?)
Treated More Than Eight Weeks						
11-15	1	1				1
16-20	1		1			1
Total	2	1	1			2

\* Patient with questionable reaction was receiving vancomycin concomitantly.

† One of the 2 patients with questionable reactions was receiving vancomycin concomitantly.

TABLE VII  
*Effect of Patients' Age and of Dosage on Toxicity of Kanamycin*

Category	No. patients	Average total dose, Gm. (range)	Average daily dose, mg./Kg. (range)	Average number days of therapy (range)	Average age (range)
Total group	106	28.2 (.5-107.9)	27.6 (6-63)	15.6 (1-84)	52.9 (12-84)
Patients with eighth nerve toxicity	22	46.7 (12-107.9)	36 (8-52)	20.3 (6-84)	50.8 (24-76)
Patients with no eighth nerve toxicity	84	23.3 (.5-104)	25.2 (6-63)	14.6 (1-66)	53.4 (12-84)
Patients with elevated creatinine due to kanamycin	12	38.8 (9.5-94.1)	29.8 (15-50)	19.1 (7-31)	64.2 (49-87)
Patients with no elevation of creatinine	94	27.2 (.5-107.9)	26.2 (6-63)	15.4 (1-84)	51.5 (12-84)

The 1 case exhibiting side effects and possible toxicity on oral kanamycin therapy has been discussed in detail in the section on tolerance.

Serial studies on patients receiving kanamycin parenterally revealed changes only in the differential blood count, urinalysis, serum creatinine, audiograms, and caloric stimulation tests.

Eosinophilia was noted in 9 patients, without any obvious accompanying sensitivity reaction.

Forty-seven patients showed urinary sediment abnormalities, but these changes were generally mild and were often noted only in one or two of the multiple specimens examined during a course of therapy. Albumin, when present, was usually graded as trace to 1 plus. Hematuria usually consisted of only occasional red blood cells per high-power microscopic field; casts, usually of the fine granular type, were present in similar numbers. Follow-up of patients showing urinary sediment abnormalities reveals that, in most cases, these abnormal findings disappeared within one week of discontinuance of therapy; that, in many patients, findings disappeared on the day treatment was stopped or within two or three days; and that, in a few patients, abnormalities persisted for two to three weeks. There was no correlation between urinary sediment changes and evidences of more important renal toxicity, such as elevation of the serum creatinine or reduction in phenol-sulfonphthalein excretion.

Twelve patients had definite creatinine elevations related to parenteral kanamycin therapy and 7 additional patients had rises that could have been due to kanamycin but also might have been related to vancomycin therapy<sup>4</sup> or other factors. Tables VI, VII, and VIII consider, among other things, the possible influence on renal toxicity of daily dose (mg./Kg. weight), total dose, duration of therapy, and

TABLE VIII  
*Toxicity in Relation to Total Parenteral Dose of Kanamycin*

Total dose, Gm.	Number of patients	Auditory toxicity	Vestibular toxicity	Elevation of creatinine	Minor urinary sediment abnormalities
15 or less	43	2 and 2(?)	1	1 and 2(?)	9
16-35	32	7	0	6	15
36-55	15	4 and 1(?)	1 and 1(?)	2 and 3(?)	12
56-107.9	16	9 and 2(?)	2 and 1(?)	3 and 2(?)	11

TABLE IX  
*Renal Toxicity: Data on Serum Creatinine*

Patient no.	Control creatinine value	Highest creatinine noted	Further rise in creatinine after drug discontinued	Number of days for creatinine to return to pretreatment range
2	1.2	2.0	None	18
20	1.5	3.1	For seven days	Still 2.8 after 25 days; lost to further follow-up
30	1.0	2.7	For three days	24
32	1.45	1.8	For two days	9
41	1.5	4.5	None	Still 2.1 after 77 days
43	1.2	2.1	None	26
45	1.0	2.6	For nineteen days	60
51	1.0	2.1	None	24
52	1.1	2.0	None	24
71	1.2	2.0	For two days	Still 1.75 after 10 days; lost to further follow-up
97	1.7	2.6	For four days	Still 2.1 after 18 days
98	1.0	2.0	None	30

age. Note that only 2 of 55 patients treated for two weeks or less had a definite rise in creatinine and that only 1 of 40 treated with 20 mg./Kg. body weight/day had creatinine elevation. Statistically, there are no significant differences between the renal toxicity group and the rest of the patients with regard to average duration of therapy ( $P = 0.1$ ), average daily dose in mg./Kg. body weight ( $P = 0.3$ ), and average total dose in Gm. ( $P = 0.1$ ). While these differences are not significant, they may represent trends toward an association, particularly in regard to total dose and duration of therapy. However, the patients in the renal toxicity group were significantly older than the patients not showing elevation of creatinine ( $P = 0.001$ , table VII). There were no instances of oliguria in this series. Table IX presents data and follow-up information on the renal

TABLE X  
*Parenteral Kanamycin: Eighth Nerve Toxicity*

Observations	No. patients
Extent of involvement audiographically	
Bilateral involvement	14
Unilateral involvement	3
No audiographic involvement	5
Sound frequencies involved, cycles	
125	4
250	4
500	4
1000	4
2000	9
4000	15
8000	14
Average number of perceptive decibels lost	
20 or less	7
21-30	2
31-40	6
>40	2
Number of 17 patients with audiographic changes also manifesting tinnitus	12
Number of eighth nerve toxicity patients with tinnitus serving as a warning of toxicity	
Of 17 patients with audiographic changes	11
Of 5 patients without audiographic changes	5

TABLE XI

*Status of Eighth Nerve Damage After Discontinuing Kanamycin Therapy*

Observations	Reactors/total patients in group
Improvement following discontinuance of drug	
Audiographic improvement	4/17
Slight audiographic improvement (related to decreased tinnitus?)	2/16
Improvement of tinnitus and/or vertigo (patients with no audiographic changes)	5/5
Further damage after discontinuance of drug	
In patients with audiographic changes	6/17
In patients with only tinnitus and/or vertigo	0/5

toxicity group patients. Note that the rises in serum creatinine are generally mild (providing therapy is promptly stopped) and that further rise in creatinine after discontinuance of drug therapy either did not occur at all or did not continue beyond one week, with one exception. This patient showed a definite, progressive rise over a period of 19 days post-treatment (three determinations weekly), with no obvious explanation other than the kanamycin therapy itself. In most instances, serum creatinines did not return to normal or pretreatment values in less than three or four weeks after treatment was stopped, and, in a few cases, elevated creatinines persisted considerably longer.

Cases showing sensitivity, possible sensitivity reactions, and possible neurotoxicity are considered in a previous publication.<sup>3</sup> One patient had diarrhea while on parenteral kanamycin, which cleared when the drug was stopped; stool cultures were negative for staphylococci and enteric pathogens, and there was no other explanation for the diarrhea.

The final and really important category of toxic reactions is the eighth nerve damage. Information on this is presented in tables V to VIII, and X to XIV. Three cases had to be considered questionable (etiologically) because of concurrent or subsequent therapy with vancomycin.<sup>4</sup> Among the 22 definite cases of eighth nerve toxicity, 5 had only tinnitus and/or vertigo without audiographic change. (Two without control audiograms had no conversational loss, and it was felt that there most likely had been no audiographic loss.) There was no significant difference between the patients with eighth nerve toxicity and those without it (table VII) with respect to average age ( $P = \text{approx. } 0.4$ ) or to average duration of kanamycin therapy ( $P = \text{approx. } 0.1$ ). There may well be a trend in that direction, however, with regard to average duration of therapy. On the other hand, there was a statistically significant difference between these two groups with respect

TABLE XII

*Extent of Audiographic Follow-up on Patients Treated with Kanamycin\**

Patient classification	Av. duration, mo. (range)	Duration of follow-up, mo.	No. of patients
Patients with eighth nerve toxicity (22)	2.6 ( $\frac{3}{4}$ – $4\frac{1}{2}$ )	Less than 1 1–2	39 22
Total patients followed audiographically (87)	1.4 (0– $6\frac{1}{4}$ )	2–3 3–4 4 or more	12 7 7

\* Total of 330 audiograms on 87 patients.

TABLE XIII  
*Relation of Eighth Nerve Damage to Dose and Duration of Kanamycin Therapy*

Daily dose, mg./Kg.	No. of patients	Audio-graphic changes	Conversa-tional loss	Over-all auditory damage			Vestib-ular damage	Impaired renal function	Duration of therapy, days
				Mild	Mod-erate	Severe			
11-15	4	2 and 1(?)	0	4	—	—	1	1 and 1(?)	15, 18, 30, 10
16-20	1	1	1	—	1	—	0	0	84
21-25	2	2	0	2	—	—	0	1	13, 25
26-30	2	1 and 1(?)	0	2	—	—	0	1	8, 14
31-40	1	1	0	—	1	—	0	1	20
41-50	11	9 and 1(?)	3*	6	3	2	3	5 and 1(?)	6, 6, 15, 15, 17, 17, 17, 19, 19, 27, 28
51-60	1	1	0	1	—	—	0	0	24

\* One patient had total deafness.

to average total dose ( $P<0.001$ ) and also average daily dose in mg./Kg. body weight ( $P = 0.001$ ), so that the larger the dose used, the more likely one is to encounter eighth nerve toxicity.

Table VI categorizes toxicity in relation to daily dose in mg./Kg. body weight, as correlated with duration of therapy. The 2 cases of auditory toxicity encountered in less than seven days (six days each) were both treated with 50 mg./Kg./day, both had impaired renal function, and both had received neomycin six months previously; they had only mild eighth nerve damage without loss of conversational hearing. The 4 cases of ototoxicity among patients treated 8 to 14 days and the 2 among those in the two or three week treatment group, who developed auditory toxicity although in the 11 to 15 mg./Kg. group, had only mild damage, with no conversational loss. Three of these 6 patients had impaired renal function.

It is noted in table X that most of the patients had bilateral involvement of the eighth nerve, and that the higher frequencies were most often involved; this is fortunate, inasmuch as 4000 and 8000 cycles are beyond the conversational threshold. There were a number of patients with large perceptive decibel losses. Most of the patients had tinnitus; this varied from a mild ringing or buzzing, brought out only by careful questioning, to severe, prolonged (months) roaring bilaterally. In most cases it was not too disturbing to the patient and did not last long. Unfortunately, 6 of 17 patients with audiographic changes did not have tinnitus, which might have served as a warning of impending further damage.

Approximately equal numbers of patients had improvement or further damage

TABLE XIV  
*Eighth Nerve Damage Following Kanamycin Therapy: Relation to Renal Function and to Previous Hearing Loss*

Observation	Reactors/total patients in group
Patients with impaired renal function (not necessarily due to kanamycin)	
Those with eighth nerve damage	9 and 2(?) / 22
Rest of group	16 / 84
Total	25 / 106
Patients with subjective hearing loss prior to kanamycin therapy	
Those with eighth nerve damage	6 / 22 (27 per cent)
Rest of group	30 / 84 (36 per cent)
Total	36 / 106

after kanamycin was discontinued (table XI). In general, changes in either direction were relatively small and occurred within one week after discontinuance of therapy, but, in some instances, the first post-treatment audiogram showing the change was taken some time after the treatment was stopped, so that the time interval is uncertain. One patient with audiographic changes (average of 20 to 25 decibel loss for three frequencies for one ear only) reversed to his pretherapy audiographic picture. The duration of audiographic follow-up is noted in table XII; a total of 330 audiograms has been taken on 87 patients to date.

Further analysis of eighth nerve toxicity, in relation to severity and various possible contributing factors, appears in tables XIII and XIV. There were 4 patients in all with conversational hearing loss (3 on very high dosage). The patients were classified as mild, moderate, or severe, depending on residual defect, loss of conversational hearing, involvement of speech frequencies, total number of frequencies affected, average number of perceptive decibels lost, and unilateral or bilateral involvement. One of the two severe cases had bilateral complete nerve deafness; the other had an average decibel loss of 30 to 35 for all frequencies but 8000 in the left ear and for three frequencies (including 2000) in the right. The other two conversational loss cases are classed as moderate. The 3 moderate cases with no conversational loss had large decibel losses (30 to 40) or involvement of several frequencies bilaterally, or both; none of the mild cases had conversational hearing loss.

The striking predisposition to eighth nerve damage in patients with impaired renal function (pre-existing or as a result of kanamycin therapy) and the lack of correlation between previous subjective hearing loss and eighth nerve damage (as a result of kanamycin therapy) will be apparent from table XIV.

There were 4 patients with vestibular damage due to kanamycin, plus 2 patients who also received vancomycin. The 4 definite cases include 2 with audiographic changes as well as 2 with only tinnitus and vertigo. One of these definite cases and 1 of the patients who received vancomycin as well are still having some difficulty five months later but appear to be gradually improving and are able to get around reasonably well. The other patients had vertigo either transiently or for a maximum of two weeks. Two of the 4 definite cases had definite impairment of caloric response.

#### DISCUSSION

The data indicate that kanamycin is an extremely effective antibiotic with a wide antibacterial spectrum, which should be useful in a variety of serious antibiotic-resistant infections. Given orally, it is an effective intestinal antiseptic and should be of use in the treatment of certain gastrointestinal tract infections.

Evidence accumulated to date indicates that microorganisms do not readily develop resistance to kanamycin in clinical usage.

Although intravenous kanamycin has been well tolerated for short periods, we feel that, on theoretical grounds, it should probably be reserved for situations in which absorption from intramuscular sites might not be reliable, inasmuch as such agents as neomycin and polymyxin may be more toxic given intravenously than when given intramuscularly.

The diarrhea noted in 1 patient receiving kanamycin intramuscularly, if actually related to the drug as it seemed to be, seems unusual, inasmuch as very little of the drug is excreted into the intestinal tract,<sup>6</sup> and there was no evidence of an intestinal superinfection.

Patient C. U., cited earlier, is of particular interest. The pseudomembranous ileitis was apparently of staphylococcal origin; the possible role of the respiratory difficulty is uncertain. In any case (whether the pulmonary problem predisposed to the gastrointestinal condition or not), it seems certain that the previous oral administration of kanamycin and its subsequent discontinuance provided an excellent background for entry of staphylococci, at a time when the kanamycin had been largely excreted, and other organisms (fecal streptococci, *E. coli*), which might have suppressed the staphylococci, had not yet grown back in. Poth<sup>8</sup> has suggested that it may be theoretically desirable to use bacitracin orally for a short while after oral therapy with neomycin is discontinued, in order to preclude the possibility of staphylococci growing in large numbers before the normal intestinal flora returns.

Although kanamycin has been effective in infections due to a wide variety of organisms, this study indicates that it is generally a poor agent for treatment of infections due to pneumococci, enterococci, *Pseudomonas*, and *Bacteroides*. It is also likely to be ineffective in infections due to streptococci, *Brucella*, anaerobes other than *Bacteroides*, and in fungus infections.

Results in the treatment of pyelonephritis due to a number of different organisms are encouraging; long-term follow-up of these cases will be important.

Considerable evidence has been presented to the effect that the ototoxicity of kanamycin is directly related to the amount of drug given. With this in mind, it is encouraging to note that the early data indicate that therapeutic efficacy is as great at a dosage level of 15 mg./Kg. body weight daily as at considerably higher dosages. Even if this should prove to be generally true, it may still be necessary or desirable to use larger doses for infections with relatively resistant organisms, for bacterial endocarditis, and for the first few days of treatment in severe infections. Contrariwise, in the presence of impaired renal function it is desirable to reduce dosage to levels lower than would be customarily used.

The possibility should be borne in mind that a patient receiving kanamycin may be more likely to develop toxicity if he has previously had such drugs as neomycin, streptomycin, vancomycin, or kanamycin itself (with or without previous toxicity); the converse might also be true. Although there is no evidence on these points, such situations should be approached with caution. Certainly none of these drugs should be used concurrently with any of the others. At the time we used kanamycin and vancomycin together, renal toxicity and ototoxicity had not yet been encountered with either agent in clinical usage.

Although considerable toxicity was encountered in this series, this study constitutes an unusually severe test for kanamycin because so many patients were treated with very large doses or for prolonged periods or both. Certainly the toxicity was minor in patients treated with 20 mg./Kg./day or less for less than two months in this series. On the basis of our experience to date, we are recommending a dosage of 15 mg./Kg./day; treatment should not be prolonged unnecessarily. We feel that it is important to use such a potent drug on a dose/weight basis rather than in terms of a fixed daily dosage, which does not consider the patient's weight.

The only major toxicity of kanamycin is its ototoxicity; the main things predisposing to such toxicity are high dosage and impaired renal function. The nephrotoxicity that we have encountered has been important only as it is predisposed to ototoxicity; it should be remembered that recovery from the nephrotoxicity may be very slow when it originally was pronounced enough to cause nitrogen retention. Older patients have a higher incidence of renal toxicity than younger ones.

The ototoxicity of kanamycin seems to be definitely less severe than that of neomycin; kanamycin appears to have the major advantage of not producing progressive deafness over an extensive period of time after the drug is stopped.

#### SUMMARY

Kanamycin is a highly effective, bactericidal antibiotic with a broad spectrum; it has been effective in a number of serious infections resistant to other agents and very likely is the most effective agent against *Proteus*. It should not be used for infections due to pneumococci, enterococci and other streptococci, *Bacteroides* or other anaerobes, or fungi. It is only occasionally effective against *Pseudomonas* and was totally ineffective in 1 case of brucellosis.

Given orally, it is poorly absorbed and therefore not indicated in systemic infections, and it is an excellent intestinal antiseptic.

Kanamycin is a toxic drug, which must be used carefully with close follow-up (including serial audiograms, when possible, and serial tests of renal function); if it is used intelligently, it should be possible to avoid major toxicity. With proper use, kanamycin will prove to be an extremely valuable drug.

Detailed consideration is given to the matter of toxicity with particular attention being paid to predisposing causes, early evidences, and means for avoiding it.

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#### BIBLIOGRAPHY

1. CRON, M. J.; JOHNSON, D. L.; PALERMITI, F. M.; PERRON, Y.; TAYLOR, H. D.; WHITEHEAD, D. F.; AND HOOPER, I. R.: Kanamycin. Characterization and acid hydrolysis studies, *J. Am. Chem. Soc.* 80:752, 1958.
2. DICKISON, H. L., AND TISCH, D. E.: A preliminary report upon the pharmacology of kanamycin. *In: Transactions of the 17th Conference on the Chemotherapy of Tuberculosis*, Memphis, Tennessee, Feb. 3-6, 1958, p. 391.
3. FINEGOLD, S. M.; WINFIELD, M.; ARONSOHN, R. B.; HEWITT, W. L., AND GUZE, L. B.: Clinical experience with kanamycin. *Ann. New York Acad. Sc.* 76:319, 1958.
4. GERACI, J. E.; HEILMAN, F. R.; NICHOLS, D. R., AND WELLMAN, W. E.: Antibiotic therapy of bacterial endocarditis. VII. Vancomycin for acute micrococcal endocarditis, *Proc. Staff Meet., Mayo Clin.* 33:172, 1958.
5. GOUREVITCH, A.; HUNT, G. A., AND LEIN, J.: Antibacterial activity of kanamycin, *Antib. & Chemo.* 8:149, 1958.
6. HEWITT, W. L., AND FINEGOLD, S. M.: Laboratory studies with kanamycin, *Ann. New York Acad. of Sc.* 76:122, 1958.
7. HUBEL, K.: Personal communication, 1958.
8. POTH, E. J.: Critical analysis of intestinal antiseptics, *J.A.M.A.* 163:1317, 1957.
9. UMEZAWA, H.; UEDA, M.; MAEDA, K.; YAGASHITA, K.; KONDO, S.; OKAMI, Y.; UTAHARA, R.; OSATO, Y.; NITTA, K., AND TAKEUCHI, T.: Production and isolation of a new antibiotic, kanamycin, *J. Antib. Ser. A.* 10:181, 1957.
10. WINFIELD, M.; CRISP, G. O.; MAXWELL, M. H., AND KLEEMAN, C. R.: Nephrotoxic effects of kanamycin. A preliminary report, *Ann. New York Acad. Sc.* 76:140, 1958.

# Kanamycin in Staphylococcal and Other Infections of Infancy and Childhood

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Kanamycin,\* a bactericidal antibiotic isolated from *Streptomyces kanamyceticus*, possesses activity against gram-negative bacilli, *Micrococcus pyogenes* var. *aureus*, and tubercle bacilli. Infectious diseases due to bacterial agents, such as the *Streptococcus*, meningococcus, and pneumococcus, respond rapidly to specific therapy. On the other hand, acute staphylococcal infections have continued to present a major therapeutic challenge. Epidemics in nurseries for the newborn and surgical wound infections due to this organism have been reported from all areas of the country. These infections that occur within the hospital environment constitute a particular problem, since the majority of "hospital strains" of staphylococci are highly resistant to penicillin and, to a lesser extent, to most other clinically useful antimicrobial agents.

In view of this situation, it is apparent that new antimicrobial agents demonstrating antistaphylococcal activity warrant careful consideration and study. It is the purpose of this study to report an evaluation of kanamycin in staphylococcal and other infections of infancy and childhood.

## MATERIALS AND METHODS

Infants and children admitted to the infectious disease division of the Children's Memorial Hospital, with confirmed or suspected infections due to *M. pyogenes* var. *aureus*, gram-negative bacilli, streptococci, and *Diplococcus pneumoniae*, were treated with kanamycin. Pre- and post-treatment cultures, urine examination, blood urea nitrogen, creatinine, liver function, and hematological studies were carried out at regular intervals. Drug susceptibility studies of organisms isolated were also performed.

A total of 36 patients, ranging in age from 3 days to 15 years old, was studied. The disease entities included pneumonia, empyema, septicemia, pyoderma, tularemia, bacillary dysentery, gastroenteritis, pharyngotonsillitis, pertussis, pyelonephritis, and suppurative otitis media. The patients were categorized according to the clinical severity of the illness.

Initially, a dosage not exceeding 15 mg./Kg./day for no longer than seven consecutive days was utilized. Later and in more serious infections, dosages of 50 to 75 mg./Kg./day were employed. In most cases the total dose was divided into two injections and given intramuscularly at 12 hour intervals. All patients were carefully followed from both clinical and laboratory aspects for the development of untoward effects to the drug. Careful attention was devoted to any auditory impairment.

## RESULTS

The patients were grouped according to clinical manifestations (table I), with

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\* The trade name of Bristol Laboratories Inc. for kanamycin is Kantrex.

TABLE I  
*Infections in Infancy and Childhood Treated with Kanamycin*

Disease	No. of patients	Bacterial isolate	Comments
Gastroenteritis	7		
	4	<i>Shigella sonnei</i>	Prompt and satisfactory response
	1	<i>Escherichia coli</i> 0111:B4	Prompt and satisfactory response
	2	None	No demonstrable effect
Respiratory infections	10		
Bronchopneumonia	2	<i>Diplococcus pneumoniae</i>	Prompt and satisfactory response
Chronic pulmonary infection	2	Mixed flora	Clinical improvement
Otitis media, suppurative	1	<i>Micrococcus, Bacillus proteus</i>	Prompt and satisfactory response
Bronchiolitis	2	None	Clinical improvement but role of drug equivocal
Tonsillitis	3	Beta-hemolytic <i>Streptococcus</i>	No effect
Pertussis	2	None	No demonstrable benefit
Tularemia	1	<i>Pasteurella tularensis</i>	Satisfactory response
Urinary tract infection	3		
	2	<i>Aerobacter aerogenes</i>	Prompt and satisfactory response
	1	<i>Pseudomonas aeruginosa</i>	Equivocal benefit

the exception of those with infections due to *M. pyogenes* var. *aureus*, who were considered separately (table II).

*Staphylococcal Infections.* Thirteen patients had infections due to hemolytic *M. pyogenes* var. *aureus*, coagulase-positive. The types of staphylococcal disease included 4 patients with pneumonia, 2 with empyema, 2 with septicemia, 1 with gastroenteritis, and 4 with pyoderma.

The results of treatment of cases of staphylococcal infection were, in general, good. One patient, a 7 month old infant with septicemia, died. However, kanamycin therapy was started relatively late in the course of the infection. The other case of staphylococcal septicemia responded promptly to kanamycin. Four cases of pneumonia and 2 cases of empyema responded satisfactorily to this agent. Staphylococcal pyoderma in 4 patients exhibited a prompt response to kanamycin.

#### CASE REPORT

*Patient 3.* This was a 4 week old white female infant (fig. 1) who developed pustular skin lesions at 1 week of age or three days following discharge from the hospital after birth. They responded to topical therapy with an antibiotic ointment, and the infant did well until four days prior to admission. At this time she developed a recurrence of pyoderma associated with redness of the skin around the umbilical stump. One day before admission respirations became labored and rapid. On examination, the infant was acutely and critically ill. The skin was covered with crops of pustular lesions, particularly in the skin folds and diaper area. There was a mild cyanosis of the hands and feet. Breath sounds were roughened and partially suppressed over the right upper lung field. A chest roentgenogram revealed consolidation in the upper half of the right lung field. Cultures of the skin, umbilicus, and blood revealed hemolytic *M. pyogenes* var. *aureus*, coagulase-positive. On admission, the patient was started on tetracycline and penicillin. On this therapy the *M. pyogenes* var. *aureus* was eradicated from the skin lesions but persisted in the blood stream. On the fourth hospital day she developed sudden increase in respiratory distress and was found to have a large right pneumothorax that required closed pleural drainage (fig. 2). This was followed by the development of pleural fluid, from which *M. pyogenes* var. *aureus* was cultured. On the third hospital day, because of the persistence of *M. pyogenes*

var. *aureus* in the blood culture, chloramphenicol had been started. Despite this therapy, the organism persisted in the blood and pleural fluid. On the seventh hospital day, kanamycin, 20 mg./Kg./day was started. Thirty-six hours later both the pleural fluid and blood cultures were sterile, and, shortly thereafter, the chest drainage was terminated and the lung re-expanded. The patient became afebrile on the tenth hospital day, and subsequently the course was uneventful. The development of a large pneumatocele is shown in figure 2. The *M. pyogenes* var. *aureus* isolated from this patient exhibited marked in vitro resistance to penicillin and tetracycline and was moderately resistant to chloramphenicol. It was susceptible to kanamycin at 1.95  $\mu$ g./ml.

#### OTHER INFECTIONS

*Gastroenteritis.* Four patients with bacillary dysentery due to *Shigella sonnei* were treated with kanamycin. There was a prompt clinical response, as manifest by drop in temperature, cessation of diarrhea, and improvement in general condition. Stool cultures promptly became negative in all 4 patients. An infant with gastroenteritis due to pathogenic *Escherichia coli* responded clinically and bacteriologically to kanamycin, after treatment with neomycin had been ineffective. One patient with staphylococcal enteritis responded promptly to therapy. Kanamycin had no apparent effect in 2 patients with gastroenteritis of nonbacterial etiology.

*Pertussis.* No significant effect could be attributed to kanamycin in 2 patients with pertussis. However, the difficulty in evaluating antimicrobial therapy in pertussis is well known, because of the variable course of this disease. While *Hemophilus pertussis* was not isolated from either case, both patients had histories of exposure, paroxysmal cough, and marked lymphocytosis. Neither patient exhibited pneumonia or other secondary bacterial complications.

*Tularemia.* An 11 year old girl with ulceroglandular tularemia exhibited im-

TABLE II  
Kanamycin in Micrococcal Infections

Disease	Patient no.	Age	Total patients	Dose mg./Kg./day	Duration of treatment, days	Comment
Pneumonia			4			
	1	2 wk.		35	5	Satisfactory response
	2	9 mo.		15	7	Satisfactory response
	3	1 mo.		20	6	Satisfactory response; see text
	4	1½ yr.		60	5	Satisfactory response
Empyema			2			
	5	9 yr.		25	7	Satisfactory response
	6	20 mo.		50	7	Satisfactory response
Septicemia			2			
	7	7 mo.		75	2	Patient died 48 hours after treatment started; see text
	8	3 days		15	7	Satisfactory response
Gastroenteritis	9	4 yr.	1	40	5	Satisfactory response
Pyoderma			4			
	10	4 days		15	5	Satisfactory response
	11	2 yr.		20		Satisfactory response; culture also revealed beta-hemolytic <i>Streptococcus</i> that persisted on therapy
	12	2 wk.		15		Satisfactory response
	13	1 yr.		15		Satisfactory response

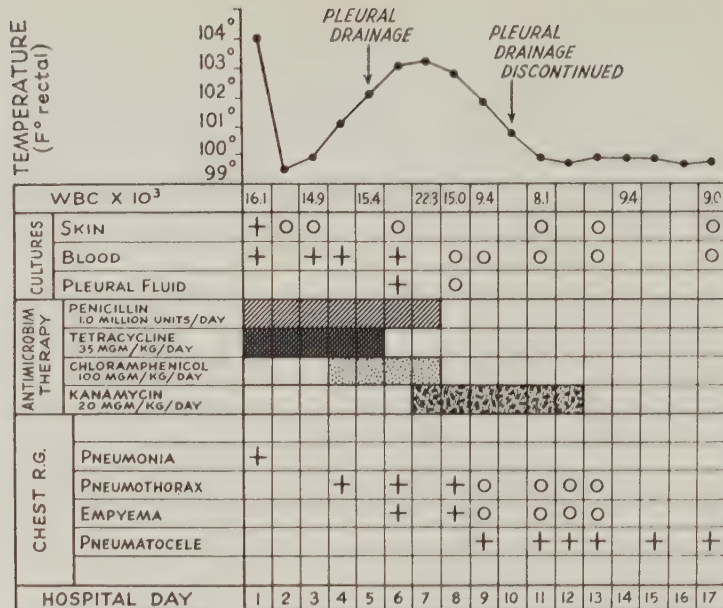


FIG. 1. This is the clinical course of T. A., a 4 week old infant with staphylococcal pneumonia and septicemia. Kanamycin was continued for 10 additional days at a dose of 10 mg./Kg. "Antimicrobim." should read "antimicrobial."

mediate improvement on kanamycin therapy, as manifest by prompt drop in temperature and healing of the draining inguinal nodes. The agglutination titer rose from 1:640 to 1:1280 while on therapy.

*Streptococcal Pharyngotonsillitis.* Three patients with pharyngotonsillitis due to group A hemolytic *Streptococcus* were treated with kanamycin. The organism was still present in throat cultures 48 hours after initiation of therapy in 2 of the 3

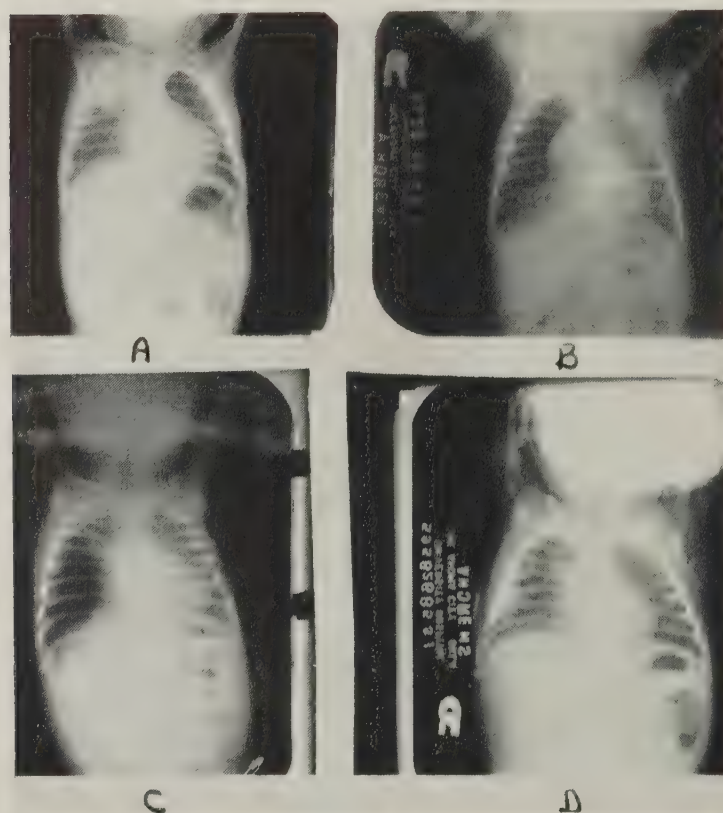


FIG. 2. Shown are serial chest roentgenograms in a patient with staphylococcal pneumonia. A, infiltration and consolidation on admission, B, depicts large pneumothorax (right); C, shows beginning development of large pneumatocele (right); D, decrease in size of pneumatocele.

patients. Another patient with a streptococcal pyoderma also responded poorly to kanamycin. Further studies of kanamycin in streptococcal infections are in progress.

*Respiratory Infections.* Several patients with respiratory disease of a nonspecific nature in regard to etiology were treated with kanamycin. Since no bacterial pathogen could be isolated from these cases, the therapeutic response was difficult to measure. The clinical response of 2 infants with bronchiolitis and 2 older children with chronic pulmonary infection of undetermined etiology was satisfactory, but the improvement could not be attributed solely to the effect of the drug, in view of the variable natural history of these diseases.

Kanamycin invoked a prompt response in 2 patients with bronchopneumonia due to *D. pneumoniae*. There was also a good result in 1 patient with suppurative otitis media, from which *M. pyogenes* var. *aureus* and *Bacillus proteus* were cultured from the aural drainage.

*Urinary Tract Infection.* The response of 2 young children with urinary tract infection due to *Aerobacter aerogenes* was bacteriologically and clinically prompt and lasting. The bacteriuria in a patient with chronic pyelonephritis due to *Pseudomonas aeruginosa* was significantly decreased with a relief of symptoms, but the urine could not be sterilized.

#### TOXICITY

No serious toxic effects to the drug were encountered. At dosages of 15 to 40 mg./Kg./day, cylinduria and albuminuria were observed intermittently. At doses greater than 40 mg./Kg./day, cylinduria, albuminuria, and slight rise in blood urea nitrogen and creatinine were observed more commonly. However, these findings were reversible on discontinuing the drug, and it was not necessary to discontinue the drug in any patient because of untoward reactions. Other reactions noted included slight eosinophilia and pain and tenderness at the site of injection. Auditory impairment, although difficult in many cases to assess because of the age of the patient, apparently did not develop in any of the treatment group. The drug was not administered for more than seven consecutive days.

#### DRUG SUSCEPTIBILITY STUDIES

In vitro susceptibility studies of many of the strains isolated were performed by the tube dilution method. While most strains of *M. pyogenes* var. *aureus* were resistant to penicillin and tetracycline and many to erythromycin and chloramphenicol, all strains tested were susceptible to kanamycin at a concentration of 5  $\mu$ g./ml. or less.

#### SUMMARY

Kanamycin was employed therapeutically in the treatment of 36 infants and children with a wide range of infections. A beneficial effect was obtained in staphylococcal infections and in bacillary dysentery. The results in urinary tract infections due to *Aerobacter aerogenes*, pneumococcal pneumonia, and tularemia are encouraging, but the number of cases treated is too small to justify final conclusions at the present. The response in a small number of patients with pertussis and group A streptococcal infections was not satisfactory. No serious untoward reactions were observed. Kanamycin appears to be a highly useful antimicrobial agent in selected cases of infections in infancy and childhood and deserves further evaluation.

# Amphotericin B: Intravenous Use in 21 Patients with Systemic Fungal Diseases

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Studies<sup>1</sup> from this laboratory on the absorption and excretion of amphotericin B showed it was poorly absorbed from the gastrointestinal tract in man, and only slight amounts were detected in serum after oral administration. These studies were paralleled by clinical trials<sup>2</sup> of oral amphotericin B therapy in patients with systemic fungal infections. Although some patients noted subjective improvement after receiving the drug orally, only 2 of 13 patients improved objectively, and in only 1 case did cultures become negative. We would now like to report our cumulative experience in the treatment of systemic mycoses by the intravenous administration of amphotericin B.

## METHODS

*Patients.* The criteria for inclusion of patients in this study were the same as those previously described.<sup>2</sup> Patients were hospitalized at the Clinical Center of the National Institutes of Health or, in four instances, at other hospitals in Washington, D. C., where the studies and treatment were directed by one of us.

*Forms of Amphotericin B Administered.* Two patients treated early in the course of this study received a suspension of amphotericin B. Nineteen patients received a soluble amphotericin B desoxycholate complex (Fungizone\*), which replaced the suspension.

*Drug Administration.* The desired dose of intravenous preparation was added to 250 or 500 ml. of 5 per cent glucose solution and given by infusion during a period of three to six hours. After a test dose of 1 to 5 mg., the desired final dosage was reached by daily increments of 5 to 10 mg. Except for 1 patient who received the drug on alternate days, patients received daily treatment. Maximum therapeutic dosage was 100 mg. daily (2.0 mg./Kg.) in 1 patient, and the range of dosage given daily to all other patients was from 0.3 to 0.75 mg./Kg.

*Clinical Studies.* Urinalyses, determinations of hemoglobin, hematocrit, white blood cell and differential counts, and liver function were performed approximately once a week. Blood urea nitrogen was determined twice a week or more frequently, if indicated. In a number of patients, repeated renal function studies (Addis counts, phenolsulfonphthalein excretion, urea and endogenous creatinine clearances) were made. A needle biopsy of the kidney was done in 1 patient. Serum amphotericin levels were determined 24 hours after infusion of the drug. The method used was that described by Louria,<sup>1</sup> with the exception of the use of the soluble preparation for the bioassay. The sensitivity to amphotericin of some of the strains of fungi recovered from the patients was determined. The concentration of amphotericin B giving 95 per cent inhibition of growth in vitro (as determined by direct hemocy-

\* The trade name of E. R. Squibb & Sons, Division Olin Mathieson Chemical Corp., for amphotericin B is Fungizone.

tometer count after 24 hours' incubation) was taken as the sensitivity of that organism.

## RESULTS

*Histoplasmosis.* Three of the 4 patients with disseminated histoplasmosis have made apparent recoveries (table I). Patient 3, previously reported,<sup>2</sup> died of his disease. Patient 2, also described before,<sup>2</sup> has now been observed for 18 months and has continued to be asymptomatic. In addition, patients 15 and 16 are culturally negative and apparently well six months after stopping therapy. It is of interest that patient 15 received a total of less than 1 Gm. of amphotericin.

*Cryptococcosis.* Of these 6 patients, 1 (patient 6) received a course of 223 mg. amphotericin B over a 15 day period (table II). No symptomatic improvement was noted during this period, and we were not able to follow the further course of her disease. Patient 17 died of his disease without apparent change in the course. In patient 9 there was reversion of spinal fluid findings to normal, and she was asymptomatic one year following the course of therapy. In patient 7, the spinal fluid protein has steadily dropped from levels of 200 mg. per cent at the initiation of treatment to 59 mg. per cent six months after stopping therapy. The cell count has dropped from 100 to 1 to 16 cells, while the cerebrospinal fluid sugar has returned to normal levels. In patient 5 there has been some objective improvement in the spinal fluid findings, but the follow-up period is short. Patient 8 had cryptococcal meningitis for eight years, with mental deterioration, cerebellar ataxia, and focal seizures. He received the drug intravenously for eight months without signs of improvement and with no change in the cerebrospinal fluid. He then received a 10 week intrathecal course of amphotericin B, supplemented by the use of the drug intravenously during the last six weeks of the course. He received intrathecally an initial dose of 1 mg. in 2 ml. of 5 per cent dextrose in water. This caused a febrile reaction, with a pleocytosis in the cerebrospinal fluid. The dose was reduced to 0.5 mg. dissolved in 10 ml. of 5 per cent dextrose in water and given twice a week for the 10 week period. Although no symptomatic or objective response was noted during therapy, remarkable improvements in his gait, balance, and memory have subsequently occurred. His spinal fluid findings have become slightly less abnormal.

*Blastomycosis.* Patient 11, previously reported,<sup>2</sup> received only 100 mg. of the drug over a 14 day period. Since the first report, her sputum cultures have become positive. The other 3 patients had disseminated disease, with extensive bone and soft-tissue involvement in 2 (patients 12 and 19) and extensive pneumonic consolidation with soft-tissue abscesses in the third (patient 18). They received relatively large amounts of drug over an extended period and have apparently recovered from their disease, both clinically and mycologically. Results are summarized in table III.

*Coccidioidomycosis.* None of the 3 patients has shown any change in cultural status after prolonged treatment (table IV). In patient 21, however, progression of disease appeared to have been slowed by therapy. In the other 2 patients, no change in clinical status has been noted.

*Candidiasis.* Patient 25 had *Candida sepsis*, which followed cystoscopy and retrograde pyelography for a left renal calculus. Six blood cultures and two urine cultures were positive for *C. albicans*. The patient received intravenously a total of 110 mg. of amphotericin B in four days. He slowly improved, and blood cul-

TABLE I  
*Intravenous Therapy of Histoplasmosis with Amphotericin B*

Patient no.	Age, yr.	Durat. illness prior to treatment, mo.	Sites of positive cultures	Sensitivity of fungus, $\mu\text{g./ml.}$	Durat. treatment, days	Maximum daily dose, mg./Kg.	Total amount of drug, mg.	Serum level, $\mu\text{g./ml.}$ (dosage, mg.)	Cultural status	Clinical status	Follow-up time, mo.
2	57	25	Blood, urine, bone marrow, ascites	—	90	60 (1.2)	5570	1.0 (60)	Neg.	Apparent recovery	18
3	1/2	1	Blood, urine, sputum, bone marrow, pleural fluid	—	15	10 (1.0)	30	—	Pos.	Dead	—
15	35	1	Bone marrow, sputum	—	15	100 (2.0)	885	—	Neg.	Apparent recovery	6
16	38	2	Bone marrow	—	85	40 (0.7)	1266	—	Neg.	Apparent recovery	4

TABLE II  
*Intravenous Therapy of Cryptococcosis with Amphotericin B*

Patient no.	Age, yr.	Durat. illness prior to treatment, mo.	Sites of positive cultures	Sensitivity of fungus, $\mu\text{g./ml.}$	Durat. treatment, days	Maximum daily dose, mg./Kg.	Total amount of drug, mg.	Serum level, $\mu\text{g./ml.}$ (dosage, mg.)	Cultural status	Clinical status	Follow-up time, mo.
5	43	54	Cerebrospinal fluid	0.06	88	30 (0.6)	1750	0.24 (25)	Unchanged (neg.)	Improved	3
6	36	15	Cerebrospinal fluid	0.07	19	20 (0.4)	223	0.48 (20)	—	—	—
7	50	25	Cerebrospinal fluid	0.06	147	80 (1.2)	2989	0.48 (40)	Unchanged (neg.)	Improved	14
8	48	64	Cerebrospinal fluid, urine	0.06	224	40 (0.6)	4227	0.48 (20)	Neg.	Improved	2
					90						
						Intrathecal 0.5 (0.007)	12				
						Intravenous 20 (0.3)	811	*			
9	37	10	Cerebrospinal fluid, urine	0.03	62	20 (0.4)	638	0.48 (20)	Neg.	Apparent recovery	13
17	77	1	Cerebrospinal fluid	—	27	50 (0.9)	621	—	Pos.	Dead	—

\* Cerebrospinal fluid amphotericin level on combined intrathecal and intravenous treatment was 0.24  $\mu\text{g./ml.}$

TABLE III  
Intravenous Therapy of Blastomycosis with Amphotericin B

Patient no.	Age, yr.	Durat. illness prior to treatment, mo.	Sites of positive cultures	Sensitivity of fungus, $\mu\text{g./ml.}$	Durat. treatment, days	Maximum daily dosage, mg. (mg./Kg.)	Total amount of drug, mg.	Serum level, $\mu\text{g./ml.}$ (dosage, mg.)	Cultural status	Clinical status	Follow-up time, mo.
11	74	36	Sputum	—	14	15 (0.2)	110	—	Pos.	Asymptomatic	16
12	45	12	Bone marrow, urine, sinus tract drainage	—	174	45 (0.6)	5185	0.24 (30)	Neg.	Apparent recovery	9
18	44	2	Sinus tract drainage	—	72	40 (0.6)	2516	0.48 (40)	Neg.	Apparent recovery	8
19	29	4	Sputum, sinus tract drainage	—	89	30 (0.5)	2298	0.12 (30)	Neg.	Apparent recovery	3

TABLE IV  
Intravenous Therapy of Coccidioidomycosis with Amphotericin B

Patient no.	Age, yr.	Durat. illness prior to treatment, mo.	Sites of positive cultures	Sensitivity of fungus, $\mu\text{g./ml.}$	Durat. treatment, days	Maximum daily dosage, mg. (mg./Kg.)	Total amount of drug, mg.	Serum level, $\mu\text{g./ml.}$ (dosage, mg.)	Cultural status	Clinical status	Follow-up time, mo.
14	81	38	Sputum	—	108	40 (0.6)	2600	2.4 (40)	Pos.	Unchanged	12
20	39	17	Sputum, synovial fluid	—	87	60 (0.8)*	1660	—	Pos.	Unchanged	2
21	25	8	Subcutaneous abscess	—	30	—	1850	—	Pos.	Unchanged	6

\* This patient was treated every other day.

TABLE V  
Intravenous Therapy of Candidiasis with Amphotericin B

Patient no.	Age, yr.	Durat. illness prior to treatment, mo.	Sites of positive cultures	Sensitivity of fungus, $\mu\text{g./ml.}$	Durat. treatment, days	Maximum daily dosage, mg. (mg./Kg.)	Total amount of drug, mg.	Serum level, $\mu\text{g./ml.}$ (dosage, mg.)	Cultural status	Clinical status	Follow-up time, mo.
22	31	2	Cerebrospinal fluid	—	60	40 (0.6)	2136	0.16 (40)	Pos.	Dead	—
23	46	1½	Blood	0.07	24	50 (0.8)	831	0.36 (50)	Pos.	Dead	—
24	40	1	Blood	0.07	—	60 (1.0)	2750	0.48 (60)	Pos.	Dead	—
25	57	½	Blood	—	4	—	110	—	Neg.	Apparent recovery	3

tures became negative. Three other patients died of their disease. Patient 23 had an endocarditis involving the left atrium and mitral valve, due to *Candida parapsilosis*, which developed following mitral commissurotomy. In patient 22, a meningeal infection due to *C. albicans* developed following the insertion of a Holter valve to relieve a noncommunicating hydrocephalus. The patient was treated intravenously with amphotericin B and given two intraventricular injections of the drug, 1 mg. in 10 ml. of dextrose in water, without benefit. The remaining patient (no. 24) had endocarditis of the mitral valve due to *C. albicans*, which developed following mitral valvulotomy. Results are shown in table V.

*Renal Function Studies.* The clinical studies on these patients will be reported elsewhere. Because of the frequency of elevated blood urea nitrogen values during therapy, other renal function studies are worthy of note. The results of urinalyses, including Addis counts, showed no change from control determinations. Similarly, no changes were noted in the phenolsulfonphthalein excretion. In a few patients there was a decrease in endogenous urea and creatinine clearance rates. The renal biopsy performed on 1 patient 10 days after cessation of therapy showed granulomas and proliferative glomerulitis.

#### TOXICITY

Side effects due to drug administration were common. Nausea, vomiting, marked anorexia, chills and fever, and mild to moderate elevation of the blood urea nitrogen occurred in a majority of patients. The elevation of blood urea nitrogen was reversible in every case, subsiding on discontinuance of the drug. Resumption of therapy at the same dosage was possible in some cases, without another rise in blood urea nitrogen. In other cases, it was necessary to reduce the dosage in order to continue therapy.

Thrombophlebitis occurred in a few patients, but it did not appear to be more frequent than with other intravenous medications. There was an apparent increase in the heart size of patient 18, without accompanying physical and electrocardiographic abnormalities. Ten days after discontinuing amphotericin B, his heart size returned to normal. Patient 8 had a generalized seizure two days following the fourth intrathecal injection of amphotericin B but subsequently received nine more weeks of intrathecal therapy without seizures. He had a history of seizures prior to treatment.

#### DISCUSSION

On the basis of these studies, amphotericin B appears to be an effective drug in the treatment of histoplasmosis. Of a total of 7 cases of disseminated disease observed by the authors in recent years, the 3 patients reported here are the first to survive. It is recommended that the drug be used intravenously for any case of severe or disseminated histoplasmosis.

Amphotericin appears to be effective also in cases of blastomycosis. Patient 12, who recovered from his illness after amphotericin therapy, had previously been treated unsuccessfully with 2-hydroxystilbamidine, although the dosage of the drug may have been suboptimal. Harrell and Curtis,<sup>3</sup> however, have reported success with amphotericin B in patients with blastomycosis in whom there had been clear-cut failures with 2-hydroxystilbamidine. It is our feeling that amphotericin is, at present, the drug of choice for disseminated blastomycosis.

The results of therapy were more difficult to evaluate in cases of cryptococcal meningitis. This was due chiefly to difficulty in determining whether active disease was present. We have observed a number of cases in which spinal fluid cultures for prolonged periods of time became negative for *Cryptococcus* on other treatment. One of these patients had survived for a time with only one abnormal finding, a depressed spinal fluid sugar level, but succumbed eventually to his disease. Others<sup>4</sup> have also reported long periods of remission. The courses of patients 7 and 9 indicate that prolonged treatment may be necessary. Especially interesting is the demonstration that amphotericin B may be given intrathecally to some patients for long periods of time without detectable ill effect. Final assessment of the effectiveness of amphotericin B in cryptococcal meningitis must await a longer period of evaluation. At the present time, of 6 of our patients, 1 has a normal spinal fluid, 1 has a near normal spinal fluid, 2 show slight objective improvement, 1 is dead, and 1 is lost to follow-up after an inadequate course of therapy. These findings do not differ significantly from those of Rubin and Furcolow.<sup>5</sup>

Our experience with amphotericin in cases of coccidioidomycosis was uniformly disappointing. These patients received 40 to 60 mg./day for periods of one month or longer. No patient was cured. Littman et al<sup>6</sup> reported cures on dosages administered every other day. These were approximately double those received by our patients.

Amphotericin was ineffective in 2 patients with *Candida* endocarditis. A recovery from this type of infection has not yet been reported. A patient with a *Candida* infection of a Holter valve and the meninges failed to respond to amphotericin. One patient with septicemia due to *C. albicans* did recover, but we do not know whether this was due to relief of ureteral obstruction or to therapy with amphotericin.

Toxic reactions occurred to some extent in all patients treated. Most commonly seen were pyrogenic and gastrointestinal reactions, which, although severe, did not necessitate stopping the drug. Some patients were able to tolerate large doses of amphotericin without developing an increase in blood urea nitrogen, while in others azotemia developed on much smaller doses. In one patient there was an increase in heart size, which receded when therapy was discontinued. In view of the report by Littman et al<sup>6</sup> of cardiac arrest occurring during the infusion of the drug, one should be alert for any cardiotoxic manifestations.

#### CONCLUSIONS AND SUMMARY

Amphotericin was used intravenously in 21 patients with culturally proved systemic fungal diseases. Diseases and number of patients treated were: histoplasmosis (4), cryptococcal meningitis (6), blastomycosis (4), coccidioidomycosis (3), and candidiasis (4). Amphotericin appears to be effective in the treatment of blastomycosis and histoplasmosis. Although some patients with cryptococcosis improved and 1 patient has apparently recovered following treatment, the evaluation of the effectiveness of amphotericin in this disease must be deferred. Although 1 patient with coccidioidomycosis improved slightly on treatment, none of the 3 has shown change in cultural status. Only 1 of 4 patients with candidiasis has thus far shown improvement. Some infected areas that were avascular (Holter valve, endocardial vegetations) were not sterilized. Toxic effects were seen to some degree in all the patients. These effects disappeared after therapy was discontinued.

#### ACKNOWLEDGMENT

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#### BIBLIOGRAPHY

1. LOURIA, D. B.: Some aspects of the absorption, distribution, and excretion of amphotericin B in man, *AM & CT* 5:295-301, 1958.
2. UTZ, J. P.; LOURIA, D. B.; FEDER, N.; EMMONS, C. W., AND McCULLOUGH, N. B.: A report of clinical studies on the use of amphotericin in patients with systemic fungal diseases. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 65.
3. HARRELL, E. R., AND CURTIS, A. C.: The treatment of North American blastomycosis with amphotericin B, *A.M.A. Arch. Dermat. & Syph.* 76:561-569, 1957.
4. BEESON, P. B.: Cryptococcal meningitis of nearly 16 years' duration, *A.M.A. Arch. Int. Med.* 89:575-583, 1952.
5. RUBIN, H., AND FURCOLOW, M. L.: Promising results in cryptococcal meningitis, *Neurology* 8:590-595, 1958.
6. LITTMAN, M. L.; HOROWITZ, P. L., AND SWADEY, J. G.: Coccidioidomycosis and its treatment with amphotericin B, *Am. J. Med.* 24:568-592, 1958.

# The Combined Use of Gamma Globulin and Broad-Spectrum Antibiotics in the Treatment of Osteomyelitis

## A Preliminary Study

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Osteomyelitis of all types continues to present many formidable problems in management. With the advent of antibiotics, there has been some improvement in the therapy of this disease. Many cases, however, have remained resistant to all types of therapy, no matter how carefully or diligently applied. The present ascendancy of the hemolytic *Staphylococcus aureus* has further increased the incidence of this disease and compounds the treatment problems for both patient and physician.

### HISTORY AND BACKGROUND

In 1952, Bruton<sup>2</sup> reported the first case of agammaglobulinemia, in which an 8 year old child lacked completely the humoral antibodies contained in the gamma globulin fraction of the serum. This resulted in severe recurrent infections of both viral and bacterial types. Effective prophylactic treatment in this and subsequent cases consisted of administration of pooled human gamma globulin. This important contribution further established the serum gamma globulin as a carrier of antibodies.

In 1954, Harris and Shick<sup>7</sup> reported on 6 children with respiratory or gastrointestinal infections that would not respond to antibiotic therapy. The addition of a small amount of gamma globulin yielded marked improvement in all instances. None of these patients had true agammaglobulinemia; these authors postulated a more subtle disturbance in the immune process, which was effectively treated by passive immunization through the use of pooled gamma globulin.

Further studies on the use of gamma globulin in bacterial infections were carried out by Barandun et al<sup>1</sup> in 1957. In that report, 15 of 23 patients with severe bacterial infections responded to pooled gamma globulin in large doses. Again, all patients had received previous appropriate and adequate antibiotic therapy with little or no result. In most instances, the gamma globulin was administered without antibiotics.

During 1957, Fisher,<sup>3</sup> through his laboratory studies, showed conclusively that small subcurative doses of chloramphenicol combined with subcurative doses of pooled human gamma globulin effectively protected mice from otherwise lethal infections of various types, including hemolytic *Staph. aureus*. This combined treatment appeared to have a synergistic effect. This author,<sup>4</sup> in a second series of mouse experiments, bolstered the evidence that the action of pooled gamma globulin is mediated through the specific antibacterial antibodies that it contains, the antibodies acting primarily, both directly and indirectly, to facilitate phagocytosis.

Waisbren<sup>9</sup> confirmed these findings in a clinical study of 46 patients. He utilized gamma globulin and antibiotic therapy for a variety of infections. In 6 patients it was definitely shown that a superior result was obtained with combined therapy than was possible with antibiotics alone. Further laboratory studies,<sup>6</sup> during 1957,

indicated a synergistic effect between ristocetin and gamma globulin in mouse infections. Knouf's results<sup>8</sup> in 10 cases of bacterial infection essentially confirmed those of Waisbren.<sup>9</sup> Several of these cases, which responded to combined therapy, had not previously responded to either antibiotics or gamma globulin when administered separately.

In the previous studies, as outlined above, a total of 8 cases of osteomyelitis was treated.<sup>1-9</sup> In each instance, a satisfactory response to combined therapy was obtained. Based on this broad foundation of clinical and laboratory evidence, the present study of 19 patients with osteomyelitis was undertaken.

#### CLINICAL MATERIAL

All clinical data are summarized in tables I to IV. Of the 19 patients studied, 17 were men and 2 were women. There were 17 white patients and 2 Negro patients in the series, and ages ranged from 8 to 42 years old. All patients were hospitalized in the Orthopaedic Service of Walter Reed Army Hospital and their management during this study was supervised directly by the authors.

The majority of patients had tibial osteomyelitis (13). Next in frequency was osteomyelitis of the upper femur involving the hip (3 patients), followed by 2 patients with femoral shaft infections, and 1 with infection of the ulna. The etiology of these infections consisted of compound wounds in 4 patients, and postoperative wound infections following various orthopedic procedures accounted for 12 cases. There was 1 acute hematogenous osteomyelitis, and 2 cases were recurrences of chronic osteomyelitis (table I).

In each case there was an open draining wound, except for the patient with acute hematogenous osteomyelitis (patient 4) and 1 of the patients with recurrent chronic osteomyelitis (patient 13). All draining wounds, prior to treatment, yielded moderate to copious amounts of purulent material in each 24 hour period. Osteomyelitis had been established for an average of 10 months, the longest period being 22 months, the shortest two months.

The diagnosis of osteomyelitis was confirmed in each case by standard roentgen-ray criteria and special sinus studies utilizing diodrast or lipidol, when applicable. All bacterial cultures were obtained deeply from within the sinus following sterile preparation of the wound edges. When a draining sinus was absent, needle aspiration under sterile conditions was utilized. Sensitivity studies were of the paper disc type. All hemolytic *Staph. aureus* in this study were coagulase-positive. Phage typing revealed the predominant strain to be 52/44a/42b/81. Hemolytic *Staph. aureus* was present in all cases except three. It was the only organism in 11 cases. In 5 patients, hemolytic *Staph. aureus* was combined with *Proteus*, *Escherichia coli*, or *Paracolonobacterium aerogenes*. In the 3 cases in which hemolytic *Staph. aureus* was not cultured, *P. aerogenes* was the only pathogen in two instances; the third case cultured *E. coli* and *Proteus* (table II).

Additional studies consisted of blood counts, sedimentation rates, and complete serum protein electrophoretic patterns, all performed initially and repeated at appropriate intervals throughout the study. The paper electrophoresis was based on a standard analytrobe method. The normal serum gamma globulin level was considered to be  $16.6 \pm 3$  per cent of total serum proteins. There was no instance of initially low gamma globulin in any of the patients. There were, however, two instances of borderline high values at the beginning of the study (patients 3 and 18, table II). Interestingly enough, these high values were present in the patients with

TABLE I  
*Clinical Data*

Patient no.	Race, sex	Age, yr.	Site of osteomyelitis	Cause of osteomyelitis	Roentgenogram findings	Duration of disease, mo.	Previous antibiotics
1	White M	29	Ulna	Fracture, open; surgical (bone graft)	Nonunion; small sequestrum	13	Chloramphenicol, erythromycin, penicillin, streptomycin
2	Negro M	35	Distal tibia and ankle	Fracture, dislocation, open	Severe disruption of ankle joint	3	Tetracycline
3	Negro M	29	Femoral shaft	Fracture, closed; surgical (nail)	Large sequestrum	12	Chloramphenicol (depression of white blood count), sulfisoxazole
4	White M	8	Mid and distal tibial	Acute hematogenous	Acute osteomyelitis	4	Tetracycline
5	White F	22	Proximal tibia	Dislocated patella; surgical	Cystic osteomyelitis	15	Chloramphenicol, novobiocin
6	White M	20	Mid tibia	Chronic hematogenous	Chronic osteomyelitis	2*	Chloramphenicol, penicillin
7	White M	26	Distal tibia	Fracture, closed; surgical	Nonunion; sequestrum	22	Chloramphenicol, oxytetracycline, penicillin
8	White M	31	Distal tibia	Fracture, open; surgical	Rush rod with parhan band	3	Chloramphenicol, erythromycin
9	White M	38	Mid tibia	Fracture, open	Nonunion	4	Chloramphenicol
10	White M	19	Mid tibia	Fracture, open	Small sequestrum	16	Chloramphenicol, penicillin, streptomycin
11	White M	42	Proximal femur and hip	Fracture, closed; surgical (prosthesis)	Prosthesis with osteomyelitis	16	Chloramphenicol, erythromycin, penicillin, streptomycin
12	White M	35	Proximal femur and acetabulum	Surgical (arthrodesis)	Nonunion	3	Novobiocin, penicillin, streptomycin
13	White M	35	Mid tibia	Chronic fracture, closed	Chronic osteomyelitis	17	Chloramphenicol, oxytetracycline
14	White M	18	Mid tibia	Fracture, closed; surgical (bone graft)	Nonunion; small sequestrum	13	Chloramphenicol, erythromycin, penicillin, streptomycin
15	White M	31	Femoral neck and hip	Fracture, closed; surgical (nail)	Sclerosis, sequestrum	12	Chloramphenicol, nitrofurantoin, penicillin, streptomycin
16	White M	22	Distal tibia	Fracture, open; surgical (nail)	Nonunion; sequestra; Lotte's nail	2	Penicillin, streptomycin
17	White M	20	Mid tibia	Fracture, open	Early union with osteomyelitis	17	Chloramphenicol, penicillin, streptomycin
18	White M	29	Femoral shaft	Fracture, closed; surgical (nail)	Large sequestra	2	Penicillin, streptomycin, oxytetracycline
19	White M	32	Distal tibia	Fracture, open; surgical	Early union; sequestra	12	Penicillin, streptomycin, tetracycline

\* Patient had disease initially 15 years prior to present study.

TABLE II  
Laboratory Data

Patient no.	Organisms isolated and sensitivities	Blood gamma globulin level	
		Initial	Final
1	Hemolytic <i>Staph. aureus</i> : chloramphenicol, novobiocin, erythromycin <i>Proteus</i> : streptomycin, chloramphenicol	19.6	21.6
2	<i>Paracolobactrum aerogenes</i> : chloramphenicol, novobiocin, erythromycin	17.6	11.5
3	Hemolytic <i>Staph. aureus</i> : chloramphenicol, novobiocin	23.0	25
4	Hemolytic <i>Staph. aureus</i> : chloramphenicol, novobiocin, erythromycin, streptomycin	14.6	
5	Hemolytic <i>Staph. aureus</i> : chloramphenicol, novobiocin, erythromycin	18.6	11.0
6	<i>Paracolobactrum aerogenes</i> : chloramphenicol, streptomycin, nitrofurantoin	18.8	10.9
7	Hemolytic <i>Staph. aureus</i> : chloramphenicol, novobiocin, oleandomycin	17.4	10.6
8	Hemolytic <i>Staph. aureus</i> : chloramphenicol, erythromycin, novobiocin	12.0	14.3
9	Hemolytic <i>Staph. aureus</i> : novobiocin, nitrofurantoin, oleandomycin	17.8	16.1
10	Hemolytic <i>Staph. aureus</i> : chloramphenicol, nitrofurantoin, erythromycin	17.7	
11	Hemolytic <i>Staph. aureus</i> : chloramphenicol, erythromycin		
12	Hemolytic <i>Staph. aureus</i> and <i>E. coli</i> : chloramphenicol, novobiocin, streptomycin, erythromycin, chlortetracycline	20.2	13.5
13	Hemolytic <i>Staph. aureus</i> : chloramphenicol, novobiocin, erythromycin, streptomycin	15.1	14.0
14	Hemolytic <i>Staph. aureus</i> and <i>Paracolobactrum aerogenes</i> : penicillin, streptomycin, chloramphenicol, novobiocin, erythromycin	17.7	17.5
15	Hemolytic <i>Staph. aureus</i> : chloramphenicol, novobiocin, nitrofurantoin; <i>E. coli</i> : nitrofurantoin	20.8	21.5
16	Hemolytic <i>Staph. aureus</i> : chloramphenicol; <i>Paracolobactrum aerogenes</i> : streptomycin	15.6	17.6
17	Hemolytic <i>Staph. aureus</i> : erythromycin, chloramphenicol, novobiocin, oleandomycin	14.9	16.8
18	<i>E. coli</i> : chloramphenicol, nitrofurantoin; <i>Proteus</i> : resistant to all	22.1	23.2
19	Hemolytic <i>Staph. aureus</i> : chloramphenicol, erythromycin, novobiocin	13.4	19.3

femoral fractures with very large sequestra. In the remaining 17 patients, the initial gamma globulin levels were essentially normal. The blood counts and sedimentation rates followed the patterns expected in infections of these types.

#### TREATMENT

All patients had been treated with antibiotics prior to this study. Thirteen received chloramphenicol in adequate dosages, singly or with other antibiotics. The remaining patients received other broad-spectrum antibiotics or penicillin in various combinations (table I).

Based on the studies of Fisher<sup>3</sup> and Waisbren,<sup>9</sup> it was decided to employ chloramphenicol as the antibiotic of choice, when applicable. The drug was used orally

TABLE III  
*Details of Therapy*

Patient no.	Dosage chloramphenicol, Gm./day	Dosage gamma globulin, ml./week	Length of treatment, weeks	Supplementary treatment
1	2.0	30	10	Cast
2	2.0	30	11	Cast
3	2.0*	30	13	Cast
4	1.0	21	9	Cast
5	2.0	30	4	
6	2.0	30	8	
7	3.0	30	16	Previous sequestrectomy
8	4.0	40	6	Removal rush rod, cast
9	2.0*	40	6	Cast
10	3.0	30	5	Cast
11	2.0	20	8	Isophane insulin
12	2.0	30	13	Nail extracted
13	3.0	30	6	Cast
14	3.0	30	10	Cast
15	3.0	40	12	Sulfisoxazole, cast
16	3.0	30	6	Sequestrectomy, streptomycin, cast
17	2.0	30	5	Incision and drainage, cast
18	2.5	30	9	Nail extracted, sequestrectomy
19	2.5	30	8	Previous sequestrec- tomy, cast

\* This patient received novobiocin instead of chloramphenicol.

in a dosage ranging from 15 to 20 mg./lb./day. Seventeen patients were treated with chloramphenicol (table III). In the 2 remaining patients, novobiocin was used orally in a dosage of 2 Gm. daily, because patient 3 had previously experienced a transient leukopenia while receiving chloramphenicol and patient 9 presented organisms that were not sensitive to chloramphenicol *in vitro*.

A 16 per cent solution of pooled human gamma globulin was employed in each case. Dosages were fixed arbitrarily at approximately 0.2 ml./lb./week (table III). The total weekly doses were administered intramuscularly in three equal parts on separate days.

The total duration of combined therapy was individualized depending on the initial and later response. It was decided that following complete clinical quiescence of the infection, a minimum of one additional week of therapy was indicated. The average duration of combined therapy for all patients was nine weeks. The shortest period was four weeks and the longest 16 weeks (table III). Additional treatment during this period consisted of immobilization of the involved part, usually by a plaster cast and sequestrectomy, or removal of metallic internal fixation in 6 patients (table III). One patient received daily isophane insulin for long-standing diabetes. Additional antibiotics were used as indicated in 2 patients. No other oral or parenteral medications were administered during the study.

TABLE IV  
*Clinical Results*

Patient no.	First indications of improvement	Time of response, wk.	Later results	Follow-up wk.	Over-all response
1	Decreased drainage No drainage	3 5	Wound remains healed	26	Excellent
2	No drainage	3	Wound remains healed	24	Excellent
3	Immediate response No drainage	5	Wound remains healed, fracture united	16	Excellent
4	No infection or bone pain	6	Quiescent	22	Excellent
5	Response No drainage	2 3	Wound remains healed	16	Excellent
6	Response No drainage	4 5	Wound remains healed	22	Excellent
7	Minimal drainage No drainage	6 12	Wound remains healed	26	Excellent
8	No drainage	2	Quiescent with early union	24	Excellent
9	No drainage	2	Quiescent with union	24	Excellent
10	No drainage	1	Wound remains healed	20	Excellent
11	No drainage	3	Slight drainage Wound remains healed	28 52	Good
12	Immediate response No drainage	9	Exacerbation, prompt closure after second course	20	Good
13	Marked response No drainage	1 10	Exacerbation Continuous slight drainage	12 16	Fair
14	Marked response	2	Slight drainage and nonunion	24	Fair
15	No improvement		Drainage	24	Poor
16	No improvement		Severe drainage	24	Poor
17	No improvement		Severe drainage	26	Poor
18	Initial decrease in drainage		Severe drainage	24	Poor
19	Initial decrease in drainage		Moderate drainage, progressive union	24	Poor

## RESULTS

Certain criteria were established for grading the results. An excellent response was characterized by prompt decrease in drainage, redness, pain, and other signs of infection, with final healing of the sinus when present and the wound remaining dry through the follow-up period, with progressive bone healing. Ten patients responded in this manner (patients 1 to 10, table IV). A good response was similar in all respects to an excellent one, with the exception of a slight transient period of drainage following cessation of therapy. This responded to a second course of treatment or spontaneously showed progressive soft-tissue and bone healing. Two are present in this group (patients 11 and 12). A fair response demonstrated an initial decrease in drainage, heat, redness, pain and progressive healing of soft tissue with, however, persistence of mild drainage and bone involvement. There was moderate over-all improvement in this group. Two fell into this category (patients 13 and 14). The patients exhibiting a poor response to treatment either did not respond or, following an initial transient decrease in drainage, promptly regressed to their pretreatment status. Five are present in this group (patients 15 to 19).

There was no relationship between the time required for initial improvement and

the chronicity of the established infection. Furthermore, no relationship could be established between chronicity and the final result. In those patients who did improve, the average time for the initial response was 10 to 21 days, manifested by a decrease in drainage, redness, heat, or deep bone pain. Several patients showed definite quiescence of the infection within two to three days (table IV). The average time for complete soft-tissue healing, when this occurred, was four and one-half weeks.

Electrophoretic serum gamma globulin levels, repeated at the end of the treatment phase, indicated a trend toward a slight decrease in those patients who responded satisfactorily, while all 5 patients who responded poorly showed an increase in their serum gamma globulin levels (table II).

No significant complications occurred as the result of the treatment. A mild erythema associated with transient pain at the site of injection was a constant finding. Patient 14 developed a nicotinic acid deficiency while undergoing treatment; this promptly cleared with appropriate therapy.

The average follow-up period for the entire series was six months.

## DISCUSSION

Considering the very short follow-up period in this series, the findings presented here can only be considered preliminary. A further, long follow-up period will be necessary for a full evaluation of combined therapy in osteomyelitis. Nevertheless, the study revealed that approximately 75 per cent of 19 patients benefited from this form of therapy. Of the 12 patients who responded to combined chloramphenicol and gamma globulin therapy, 9 had had previous adequate trials of chloramphenicol alone without benefit.

A comparison between the failures and satisfactory results indicated certain differences. The wounds in patients who responded, cultured, in most instances, a single organism, sensitive to one or more antibiotics used in this series. The wounds of 3 of the 5 failure cases cultured mixed organisms, which consisted of relatively resistant *E. coli.*, paracolon bacilli, and/or *Proteus*. In addition, 4 of the 5 failure cases exhibited gross sequestra, demonstrated by standard roentgen-ray techniques. These findings substantiate those of Fisher,<sup>5</sup> who suggests that combined therapy will only work synergistically when bacteria are sensitive to the antibiotics used and the disease is, of course, accessible to phagocytes, antibodies, and drugs. It is presumed that the dead bone in those cases with sequestra acted as a barrier, preventing satisfactory utilization of antibodies, phagocytes, and antibiotics.

Although further clinical and laboratory experiments will be required to determine its lasting value, combined therapy for selected cases of osteomyelitis appears promising.

## SUMMARY

A preliminary study of 19 patients with various forms of active osteomyelitis is presented. These patients were treated with large doses of pooled human gamma globulin and chloramphenicol in 17 cases and gamma globulin with novobiocin in 2 cases. The patients' response to therapy was considered excellent in 10, good in 2, fair in 2, and poor in 5, with 75 per cent showing some improvement. Of the 12 patients who responded to chloramphenicol and gamma globulin therapy,

9 had an adequate prior trial of chloramphenicol alone without response. Factors causing poor results were gross sequestra or mixed infections with relatively resistant bacteria. There were no significant complications. This type of combined therapy shows promise in selected cases of osteomyelitis.

#### BIBLIOGRAPHY

1. BARANDUN, S.; KIPFER, R.; RIVA, G., AND NICOLET, A.: The therapeutic application of gamma globulin in bacterial infections, *Schweiz. med. Wchnschr.* 87:155, 1957.
2. BRUTON, O. C.: Agammaglobulinemia, *Pediat.* 9:722, 1952.
3. FISHER, M. W.: Synergism between human gamma globulin and chloramphenicol in the treatment of experimental bacterial infections, *Antib. & Chemo.* 7:315, 1957.
4. FISHER, M. W., AND MANNING, M. C.: The specific antibody nature of the therapeutic action of gamma globulin in experimental bacterial infections in mice. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 572.
5. FISHER, M. W.: Panel discussion. Host resistance and chemotherapy. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 1033.
6. HOLPER, J. C.; RICKHER, C. J., AND SYLVESTER, J. C.: Ristocetin: Effect of gamma globulin on in vivo activity. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 577.
7. HARRIS, J. R., AND SHICK, B.: The use of gamma globulin in infections refractory to antibiotics, *J. Mt. Sinai Hosp.* 21:148, 1954.
8. KNOUF, E. G.: The combined use of gamma globulin and chloramphenicol in the treatment of refractory infections. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 585.
9. WAISBREN, B. A.: The treatment of bacterial infections with the combination of antibiotics and gamma globulin, *Antib. & Chemo.* 7:322, 1957.

Extensive experiments have been conducted to explore the potentialities of antibiotics as food preservatives. In November, 1955, the Food and Drug Administration approved the use of chlortetracycline for the preservation of raw poultry, and about one year later similar approval was given for oxytetracycline. Tolerance levels for these drugs were established under the provisions of the Miller Pesticide Chemicals amendment to the Food, Drug, and Cosmetic Act, passed by the 83rd Congress in 1954. These drugs act as preservatives and extend the "shelf life" of the fowl. Under the Miller amendment, the applicant must demonstrate "usefulness" to the satisfaction of the Department of Agriculture and "safety" to the Food and Drug Administration.

The tolerance level for both chlortetracycline and oxytetracycline in the raw bird as it moves in interstate commerce was established at 7 parts per million in any part of the bird. Before approval of this tolerance level was granted, it had to be shown that no significant antibiotic residues could be found in the poultry after cooking by broiling, frying, boiling, or baking. Under consideration now is the use of chlortetracycline and oxytetracycline as preservatives for fish as a means of extending "shelf life." Unfortunately, even though smaller amounts of these antibiotics are used for preservation of fish than for poultry, we have been unable to show that ordinary methods of cooking treated fish (broiling, frying, boiling, or baking) will invariably eliminate the residual antibiotic. Furthermore, some fish are eaten raw, smoked, or pickled, and in all these cases the consumer might well ingest antibiotic residues. Although both antibiotics are approved in Canada for use in the preservation of fish, before tolerance levels for them can be established in the United States it will be necessary to demonstrate that the residues found are not dangerous to the public health.

Although sensitivity to the tetracyclines is extremely rare, it does occur, and the question naturally arises as to whether these unfortunate individuals might react to even those small residues (on the order of  $0.4 \mu\text{g./Gm.}$ ) found in some treated or cooked fish. It was to test this possibility that the present study was undertaken. Because of the extreme difficulty of locating patients with a known history of sensitivity to the tetracyclines, only 7 could be included in this study. Two of the patients were personally studied by one of us, while the other 5 were studied by 5 other investigators (1 patient each) scattered throughout the country.

At the beginning of the study, identical protocols were established so that each physician participating in the study would conform to the same general outline. The protocol included an allergic history of each patient (including childhood allergies), the causes of the allergies, and also a history of allergy of members of the family. Each patient was questioned as to his sensitivity to the tetracyclines to determine to which of the three, i.e., chlortetracycline, oxytetracycline, and tetracycline, he had previously exhibited a reaction. Inquiry was made also as to the dose used, the method of administration, and the physician who prescribed the drug and who witnessed or treated the reaction. The type of reaction and symptoms were also carefully noted. Thus, every precaution was taken to insure that these patients were

truly sensitive to one of the tetracyclines. A physical examination was performed on each patient before starting the study.

#### EVIDENCE OF SENSITIVITY

*Case 1.* R. N. (W. M.) had a severe reaction in March, 1958, one-half hour after ingestion of a 250 mg. capsule of tetracycline. This consisted of dyspnea, tachycardia, lacrimation, and erythema of the face. A similar reaction occurred after taking 5 ml. of a tetracycline syrup (125 mg.). He was treated with an antihistamine and recovered within a few hours. This patient is a laboratory worker and is also sensitive to rabbit dander and dust.

*Case 2.* M. S. (W. F.) was given chlortetracycline, 250 mg., in October, 1956. After the third dose she developed generalized urticaria, erythema, angioneurotic edema, conjunctival injection, and pruritus. Symptoms subsided after a few hours without treatment.

*Case 3.* A. W. (W. M.), a physician, reacted to a 250 mg. capsule of chlortetracycline with pruritus, urticaria, and erythema multiforme. The eruption faded with increased pigmentation. Subsequently, after a 250 mg. dose of oxytetracycline, the erythema multiforme appeared in the same spots as previously and faded again with increased pigmentation.

*Case 4.* I. S. (W. M.), in 1950, took 250 mg. chlortetracycline and reacted with pruritus, urticaria, and erythema. Similar reactions occurred with oxytetracycline in the same dosage.

*Case 5.* E. S. (W. M.) was given therapeutic doses of chlortetracycline following which he reacted with angioneurotic edema and urticaria. This patient fails to show a reaction following three days of therapeutic doses of oxytetracycline but does react if the dose is continued on to the fourth or fifth day.

*Case 6.* R. F. (W. F.) was administered 250 mg. of chlortetracycline (1955) and 20 minutes later exhibited angioneurotic edema of tongue, lips, and cheek, and also had dysphagia. Three months later she had a similar reaction following ingestion of 250 mg. of oxytetracycline and six months later had the same reaction after 250 mg. of tetracycline.

*Case 7.* P. D. (W. F.) took a 250 mg. chlortetracycline capsule (1952) and reacted with abdominal pains and diarrhea. In 1956, she was given 250 mg. of tetracycline. In a few minutes she developed pruritus and, later, diffuse urticaria.

#### METHOD

Capsules were made containing 0.5, 1.0, 5.0, and 25.0 mg. of chlortetracycline and also placebos consisting of milk sugar, identical in appearance. It should be noted that these doses of chlortetracycline are from 6 to 300 times that found occasionally in an average serving (one-half pound) of cooked or processed fish. The double-blind method was used, whereby neither the physician nor the patient knew which capsule was used on a given day.

The capsules were put in random order, although this was done so that each subject invariably was administered increasing doses of chlortetracycline, from 0.5 to 25.0 mg. Each patient was given eight capsules: four placebos and the four doses of chlortetracycline. The order of administration followed a standard table of random numbers. Capsules were administered on successive days, and the patient was kept under observation for two to three hours after administration. He was then examined for objective and questioned for subjective evidence of a reaction. Six of the 7 patients exhibited absolutely no reaction to any of the capsules. One patient experienced a slight amount of itching two hours after the 1.0 mg. dose. This lasted approximately 15 to 20 minutes. There were no objective signs of a reaction. Furthermore, this patient showed no reaction whatsoever following subsequent doses of 5.0 and 25.0 mg. Table I shows the method of random selection. It was known only to the person charged with selecting the random order of the capsules.

TABLE I

*Method of Random Selection of Placebos and Increasing Doses of Chlortetracycline*

Patient no.	Order of administration							
	1	2	3	4	5	6	7	8
1	P*	P	0.5†	P	1.0	5.0	P	25.0
2	0.5	1.0	5.0	25.0	P	P	P	P
3	P	P	0.5	P	1.0	P	5.0	25.0
4	0.5	P	1.0	5.0	P	25.0	P	P
5	P	0.5	P	P	1.0	P	5.0	25.0
6	P	0.5	P	1.0	5.0	P	25.0	P
7	0.5	1.0	P	5.0	P	25.0	P	P

\* Placebo.

† The figures indicate milligrams of chlortetracycline hydrochloride.

A duplicate study on the same subjects and with the same observers has been completed with oxytetracycline. A similar but not identical table of random doses was used. In this study, 6 of the 7 subjects again showed no reaction to the placebo or oxytetracycline capsules.

The seventh subject, within an hour of taking the 25.0 mg. dose of oxytetracycline, exhibited nasal stuffiness, sneezing, and lacrimation. However, he was not the same one who previously complained of itching following a 1.0 mg. dose of chlortetracycline. (That subject later was given 5.0 and 25.0 mg. of chlortetracycline without difficulty.)

The subject reacting in the oxytetracycline group, a laboratory worker, is not only sensitive to the tetracyclines but to rabbit dander, dust, and, probably, to certain of the pollens. Because the reaction observed in this subject after the 25.0 mg. dose might have been due to other allergens contacted during his daily work, this dose was repeated after he had completed his series of eight capsules. He was told to avoid the animal room areas and contact with operations where he might encounter antibiotic dust. Following ingestion of the second 25.0 mg. dose of oxytetracycline, an allergic reaction again occurred similar to the first one. The following day, in order to check the previous negative result with chlortetracycline, he was given a 25.0 mg. capsule of this drug. No reaction occurred. One week later he was given 30.0 mg. of this drug and again no reaction occurred. The following day a dose of 50.0 mg. of chlortetracycline failed to elicit a reaction.

## DISCUSSION

Sensitivity to the tetracyclines is rare. In 7 cases of documented sensitivity to therapeutic doses of one or more of the tetracyclines, only 1 showed a slight subjective reaction to 1.0 mg. of chlortetracycline. The pruritus claimed by this subject was mild and transient and was classified as psychosomatic, since no such reaction or any other allergic manifestations were evident after subsequent doses of 5.0 and 25.0 mg. Furthermore, this subject exhibited no reaction to 0.5, 1.0, 5.0, and 25.0 mg. doses of oxytetracycline.

In the case of the laboratory worker (case 1), nasal stuffiness, sneezing, and lacrimation occurred after the 25.0 mg. dose of oxytetracycline and again a few days later after a repeat check dose of 25.0 mg., while no reaction occurred in the intervening days on placebos.

Of considerable interest, however, is the fact that this patient did not exhibit a reaction to 25.0 mg. of chlortetracycline initially nor on a repeat test with this dose.

Because of this anomaly the subject was given 30.0 mg. and later 50.0 mg. of chlortetracycline, and both failed to produce a reaction, indicating that this subject is at least considerably less sensitive to chlortetracycline than to oxytetracycline.

From a review of the brief case histories of the 7 subjects under study, it will be noted that only 2 are known to be sensitive to a single tetracycline, while 4 are sensitive to two of the tetracyclines, and 1 is known to be sensitive to all three. As a matter of fact, however, it is usually assumed that a subject sensitive to one of the tetracyclines is likely to be sensitive to the others as well.

Apparently there are differences in degrees of sensitization of an individual to the tetracyclines, as exemplified in case 5. This young white man reacts with a single capsule of chlortetracycline but has been administered therapeutic doses of oxytetracycline for up to three days without exhibiting a reaction, showing evidence of sensitivity to this drug only on the fourth or fifth day. This difference in degree of sensitivity to one of the tetracyclines as compared to another is emphasized by case 1 who reacts promptly to 25.0 mg. of oxytetracycline but fails to react to 25.0, 30.0, or even 50.0 mg. of chlortetracycline. (This subject is sensitive to therapeutic doses of tetracycline.)

These data support the thesis that there are sufficient differences in the molecular structure of the tetracyclines to separate them antigenically at least in some sensitive individuals. It appears also that the demonstration of antigenic differences among sensitive subjects is related to the degree of sensitivity of the individual to the tetracycline involved.

#### SUMMARY

In a double-blind study, as much as 25 mg. of chlortetracycline was administered to 7 subjects sensitive to therapeutic doses of one or more of the tetracyclines without reaction. Except for 1 of these subjects, a similar result was obtained with up to 25 mg. of oxytetracycline. This individual, who was known to be sensitive to therapeutic doses of tetracycline, reacted with lacrimation, stuffiness, and sneezing following administration of 25 mg. of oxytetracycline but, on retesting, failed to show a reaction to as much as 50.0 mg. of chlortetracycline.

# The In Vitro Inhibition of Staphylococcal Penicillinase by Various Compounds of Chemotherapeutic Potential

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The therapeutic use of penicillin is limited greatly today by the finding of penicillin-resistant organisms as etiological agents in over 50 per cent of staphylococcal infections of hospital inpatients.<sup>1-4</sup> Were it not for this resistance, penicillin, because of its low toxicity and high bactericidal activity in relatively low concentrations, would still be the antibiotic of choice for treatment of staphylococcal disease.<sup>5</sup>

Penicillin-resistant staphylococci encountered in clinical infections have the common characteristic of producing the enzyme penicillinase,<sup>6</sup> which can destroy penicillin by degrading it to penicilloic acid;<sup>7</sup> it is by virtue of penicillinase that these strains of staphylococci are resistant to relatively high concentrations of the antibiotic.<sup>2, 8-11</sup> Staphylococcal penicillinase has been found both intracellularly (or closely bound to the bacterial cell<sup>8, 12-16</sup>) and extracellularly.<sup>13, 17-19</sup>

If a penicillinase inhibitor were found that could be concentrated in the human host to an effective but nontoxic level, it is postulated that penicillin, administered simultaneously with the inhibitor, might then prove to be an effective controlling agent of penicillin-resistant staphylococcal disease. It has been theorized that such an inhibitor, to be fully effective in potentiating the antibacterial action of penicillin, should be capable of penetrating into the bacterial cell, so as to inactivate penicillinase at its site of production, as well as extracellularly.<sup>13, 20</sup>

Antipenicillinase sera were found to protect penicillin from destruction by penicillinase preparations in vitro<sup>13, 21-23</sup> and from extracellular penicillinase elaborated in vivo.<sup>23</sup> However, biological assays indicated that although antipenicillinase rendered cultures of certain penicillin-resistant bacteria somewhat more sensitive to the action of penicillin, the cultures still were appreciably resistant to the antibiotic. It was theorized that although antibody antipenicillinase inactivated extracellular penicillinase and left the bacteria more sensitive to the action of penicillin, bacterial cellular membranes probably were impermeable to the immune protein, and antipenicillinase was prevented, thereby, from exerting its activity against intracellular penicillinase.<sup>13</sup>

Investigators<sup>20, 21, 24-28</sup> have examined a wide variety of compounds for their potency as inhibitors of the penicillinases formed by various genera of bacteria. In this study, we have assayed the ability of certain of these compounds, selected because of their chemotherapeutic potential, to inhibit staphylococcal penicillinase. The following compounds were tested: quinine, quinacrine, chloroquine, trypsin, chymotrypsin,  $\alpha$ -diethylamino-2,6-aceto-xylicide (Xylocaine), sulfathiazole, sulfadiazine, sulfanilic acid, and D-penicillamine. Studies of the penicillin-sparing effects of these compounds have been performed by manometric and iodometric titration techniques; bacteriological turbidity growth assays have indicated the capacity of the penicillin inhibitors to potentiate the action of penicillin against penicillin-resistant strains of *Staphylococcus aureus*.

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*Penicillinase Preparation.* The enzyme was prepared by a modification of the method used by Váczi and Uri,<sup>28</sup> from *Staph. aureus* VCH 8 R, isolated from a patient with staphylococcal pneumonia. The organism was coagulase-positive, of phage type 42B/52A/80/81/VA4, and resistant to 100 u./ml. of penicillin. The culture was grown on brain-heart infusion agar at 37 C. for 24 hours. After this time, the growth was washed off with physiological saline and centrifuged. The packed cells were then washed and centrifuged three times with chilled physiological saline. Seven volumes of acetone (at -20 C.) were added to each volume of packed cells. This suspension was maintained at -20 C. for two hours, then centrifuged, and the supernatant discarded. The cold acetone extraction was repeated three times. Following this, ethyl ether, cooled to -20 C., was added in a 7:1 ratio to the bacterial mass and the mixture maintained at -20 C. for one hour. Following centrifugation and decanting, the cold ether procedure was repeated twice. After final centrifugation, the extracted cells were rapidly dried under vacuum and then ground to a white powder in a mortar. This one batch of powder, maintained under desiccation in the refrigerator, was used throughout these experiments as the source of penicillinase. One mg. of the penicillinase powder inactivated 2808 u. of penicillin G.

*Penicillinase Assay. IODOMETRIC METHOD.*<sup>29</sup> Twenty thousand units of penicillin G, dissolved in 20 ml. phosphate buffer at pH 7.0, were added to test flask (A) and control flask (B). Varying concentrations of the penicillinase preparation were added to a series of A flasks. All flasks were incubated at 37 C. for one hour. Into both the A and B flasks were placed 0.5 ml. of 1 *N* hydrochloric acid and 4.0 ml. of a 0.1 *N* iodine in potassium iodide solution. In addition, penicillinase concentrations equal to the amounts originally placed in A flasks were added to the B flasks. After a 5 minute incubation period at room temperature, the excess iodine in both sets of flasks was titrated with a 0.1005 *N* sodium sulfite (standardized against potassium iodate), using a few drops of a 1 per cent soluble starch indicator. The end point was reached when the solution first became colorless. To calculate the number of penicillinase units in any particular enzyme preparation, the volume (ml.) of sodium sulfite utilized by the control (B) minus the volume used in titrating the test flask (A) was multiplied by the normality of the sodium sulfite solution (0.1005) and then by the factor 1073. According to this method, 1 u. penicillinase inactivates 107.8 u. penicillin G. All concentrations of penicillinase and control flasks were run in triplicate. By this assay method, our penicillinase preparation showed 26 u./mg. penicillinase.

*IODOMETRIC METHOD (WITH INHIBITOR PRESENT).* In these studies, there was an initial incubation period of 30 minutes at 37 C. for the mixture of penicillinase (4 or 8 mg./flask) and the particular concentration of the inhibitor being tested, prior to the addition of the penicillin. The remainder of the test was as described in the iodometric method. In the case of each inhibitor, tests were performed to determine whether the inhibitor itself had any effect on the penicillin. Such tests were invariably negative.

*MANOMETRIC METHOD.*<sup>21</sup> Penicillinase titrations were carried out in standard Warburg flasks calibrated for a total volume of 3.2 ml. To the sidearm of each flask was added 0.2 ml. penicillin G, 20,000 u., and, to the main chamber, 3.0 ml. of various concentrations of the penicillinase preparation dissolved in 0.0145 *M* sodium bicarbonate. Control flasks with just penicillin and bicarbonate solution

were run simultaneously. All vessels were thoroughly gassed with a 95 per cent nitrogen-5 per cent carbon dioxide mixture prior to mixing the contents of the vessel. A pH of 7.4 was obtained after mixing. The following penicillinase concentrations per flask were run in triplicate: 0 to 1 mg. (in 0.1 mg. steps) and 1 to 8 mg. (in 1 mg. steps). It was found that the  $\mu$ l. of carbon dioxide produced increased linearly with increase in penicillinase concentration in the 0.1 mg. to 3.0 mg. range. Then, using the method of least squares, a standard curve for microliters of carbon dioxide produced by penicillinase concentrations within this range was established. All further experimental values for penicillinase activity were corrected by reference to this standard curve. Under the test conditions, 1.0 mg. of the penicillinase preparation used throughout this study produced 48  $\mu$ l./hr. carbon dioxide.

**MANOMETRIC METHOD (WITH INHIBITOR PRESENT).** In these experiments, penicillinase (1 mg. per flask used throughout) was shaken with the particular concentration of the inhibitor being tested for 30 minutes at 37 C. in the main chamber of the Warburg vessel. The penicillin was then tipped in from the sidearm. Controls without inhibitor present were run simultaneously. The difference between carbon dioxide production without inhibitor and with inhibitor present was assumed to represent the extent of penicillinase inhibition by the specific agent in the concentration being tested. Here, also, evidence for any direct effect of concentrations of inhibitor on penicillin activity (carbon dioxide evolution from sodium bicarbonate) was sought but not found for any of the inhibitors.

**Preparation of Inhibitor Solutions for Penicillinase Assay.** Inhibitor solutions were made up in distilled water just prior to use. Trypsin and chymotrypsin were adjusted to pH 8.0. Quinine solutions were adjusted to pH 6.9. Quinacrine, chloroquine,  $\alpha$ -diethylamino-2,6-aceto-xylidide, sodium sulfanilate, sulfathiazole, and sulfadiazine were all adjusted to pH 7.4. Due to added buffers in both the iodometric and manometric procedures, all reaction mixtures were at approximately pH 7.4.

**Bacterial Cultures.** Fifteen strains of *Staph. aureus*, isolated at Variety Children's Hospital from various infectious processes, were assayed initially for penicillin sensitivity by the antibiotic disc plate procedure. Twelve of the strains were coagulase-positive penicillin-resistant; each of the 12 strains, by studies utilizing the satellite growth technique,<sup>30,31</sup> was found to produce penicillinase.

**Microbiological Assay for Penicillinase Production.** In the modified procedure employed,<sup>31</sup> an inoculum of *Sarcina lutea* plus penicillin, diluted to give a final test concentration of 0.005 u./ml., were added to melted infusion agar cooled to 45 C. Following solidification of the agar, an inoculum of the staphylococcal culture to be assayed was spread over a 1 sq. cm. area of a quadrant of the plate. Thus, a minimum of two test staphylococcal cultures and a positive and negative staphylococcal control could be assayed per plate. After 48 hours of incubation at 37 C., penicillinase production by the staphylococci was indicated by growth of satellite colonies of *Sarcina* in the agar zone directly under and about the surface staphylococcal growth.

**Turbidimetric Assay for Bacterial Sensitivity to Penicillin Plus Inhibitors.** A modified tube dilution technique<sup>32</sup> was used for these determinations. Assays were performed in nutrient broth (BBL) prepared at 1½ strength, at pH of 7.0. However, for determinations of the effects of trypsin, the medium was buffered with phosphate and adjusted to pH 7.4.

Stock solutions of crystalline potassium penicillin G were prepared with sterile distilled water and stored in the deep freeze at -20 C., where they maintained

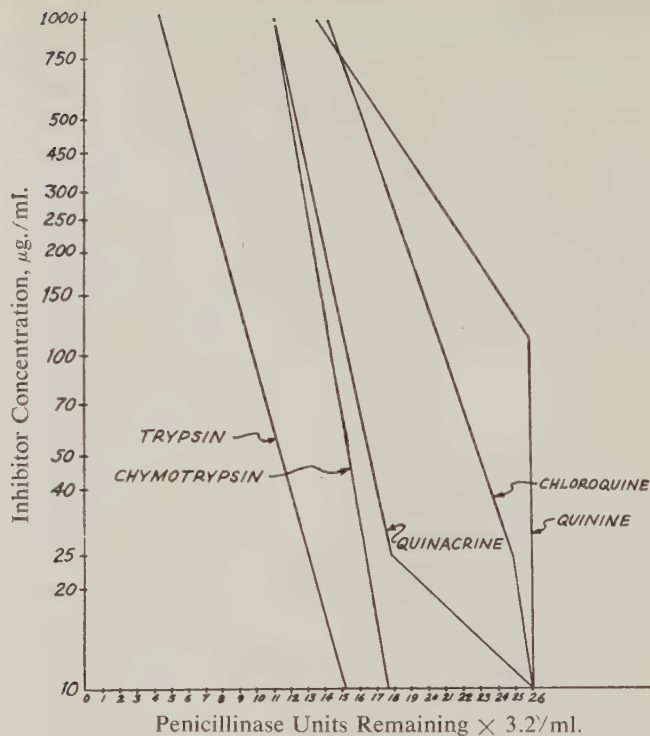


FIG. 1. Manometric measurement of penicillinase inhibition by trypsin, chymotrypsin, quinine, quinacrine, and chloroquine. Activity was measured for 1 hour at 37 C. Inhibitor concentrations are plotted logarithmically. Each Warburg flask had an initial concentration of 26 penicillinase units contained in a final volume of 3.2 ml.

their potency for a period of at least one month. Working solutions of penicillin were prepared from the thawed stock solutions at the time each test was performed.

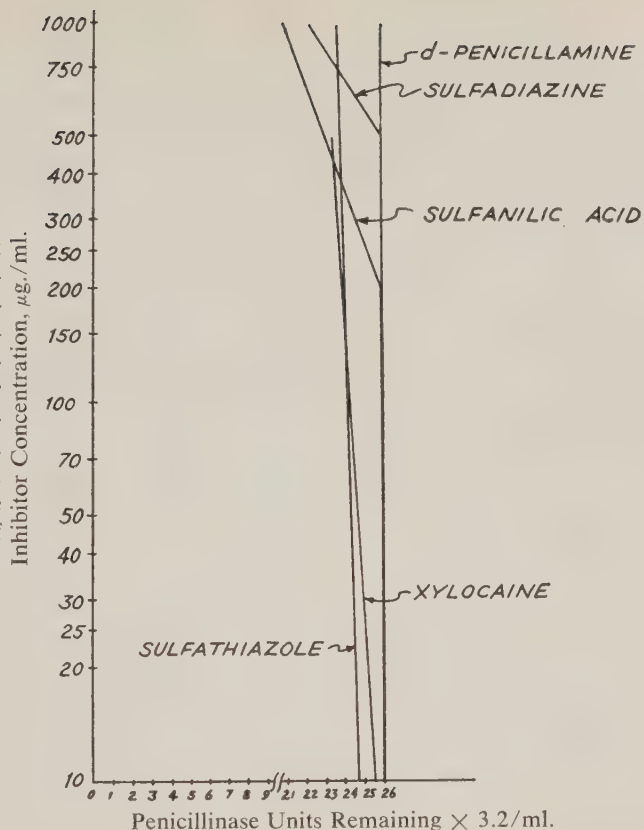
All inhibitor compounds, with the exception of trypsin, were prepared in stock aqueous solutions, adjusted to pH 7.0, filtered through ultrafine fritted glass filters for sterility, and used either immediately or after being kept overnight under refrigeration. Penicillinase-inhibitor compounds were assayed at final concentrations of 10, 25, 100, 500 and 1000  $\mu\text{g./ml.}$  Trypsin solutions were prepared similarly; however, they were set at pH 7.4 and were used immediately after preparation, for potency of trypsin solutions was found to diminish markedly following overnight storage at 5 or  $-20^\circ\text{C.}$  Trypsin was assayed at final concentrations of 93.8, 187.5, 375, 750, 1500, and 3000 u./ml.

The general assay procedure consisted of the preparation of a series of tubes, each containing 0.1 ml. of a freshly prepared aqueous dilution of penicillin in a desired concentration. To each of the tubes was added 3.8 ml. of broth and 1.0 ml. of aqueous solution of the inhibitor compound. Bacterial inocula, consisting of 0.1 ml. of a 1:10 dilution in fresh broth of an 18 hour nutrient broth culture of the assay staphylococcal culture, were then added, bringing the total volume of each tube to 5 ml. Control tubes, lacking either penicillin or inhibitor compounds, were brought to equal volume with sterile distilled water. Contents of the tubes were mixed and the tubes incubated at 37 C. The assay end point for each titration series, utilizing a fixed concentration of penicillin combined with varying concentrations of an inhibitor compound, was considered to be the lowest concentration of penicillinase-inhibitor compound that prevented visible growth after a given period of incubation.

## RESULTS

The abilities of 10 different compounds to inhibit staphylococcal penicillinase

FIG. 2. Manometric measurement of penicillinase inhibition by D-penicillamine,  $\alpha$ -diethylamino-2,6-aceto-xylidide (Xylocaine), sulfanilic acid, sulfadiazine, and sulfathiazole. Activity was measured for 1 hour at 37 C. Inhibitor concentrations are plotted logarithmically. Each Warburg flask had an initial concentration of 26 penicillinase units contained in a final volume of 3.2 ml.



are compared in figures 1 and 2. The measurements in these studies were made in the Warburg apparatus for a period of one hour at 37 C. Each Warburg flask had an initial total concentration of 26 penicillinase units (1 mg. of the staphylococcal extract) contained in 3.2 ml. The manometric assay method depends on the fact that, within relatively small enzyme concentration levels, there is a linear relation-

FIG. 3. Iodometric measurement of penicillinase inhibition by trypsin, chloroquine, and quinine. Activity was measured for 1 hour at 37 C. Inhibitor concentrations are plotted logarithmically. Each flask had an initial concentration of 104 penicillinase units contained in a final volume of 5 ml.

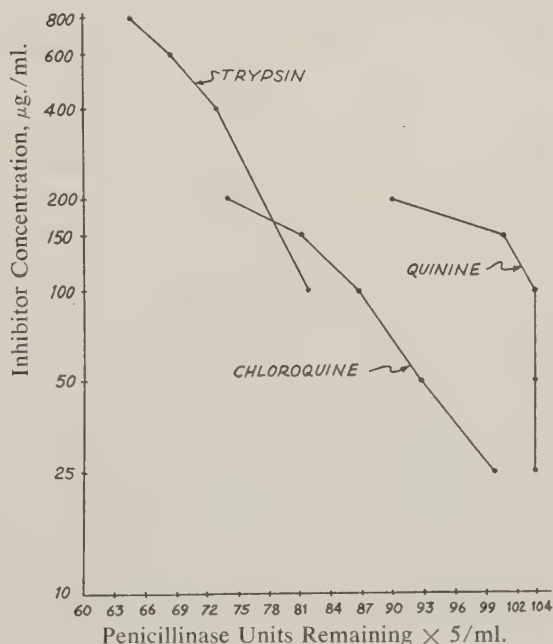


TABLE I

*Sensitivity of Penicillinase-Producing Staphylococcus aureus VCH 8 R to Penicillin Plus Quinine, Quinacrine, Chloroquine, or Trypsin*

Inhibitor	Hours of incubation at 37 C.	Penicillin concentration, u./ml.						
		0	0.01	0.1	1	5	10	100
Quinine†	4	500*	100	10	0	0	0	0
	8	500	500	500	100	25	0	0
	24	500	500	500	500	500	500	100
	48	500	500	500	500	500	500	100
Quinacrine†	4	25-100	25	25	0	0	0	0
	8	25-100	25-100	25-100	10	10	0	0
	24	25-100	25-100	25-100	25-100	25-100	25-100	10-25
	48	500	100	100	100	100	100	25
Chloroquine†	4	1000	1000	1000	0	0	0	0
	8	1000	1000	1000	500	500	0	0
	24	>1000	1000	1000	500	500	500	100
	48	>1000	>1000	1000	500	500	500	500
Trypsin‡	4	93.8	93.8	93.8	93.8	0	0	0
	8	1500	750	375	187.5	93.8	93.8	0
	24	>3000	>3000	>3000	>3000	>3000	3000	93.8
	48	>3000	>3000	>3000	>3000	>3000	>3000	>3000

\* The lowest concentration of inhibitor giving complete growth inhibition in the presence of the indicated concentration of penicillin is recorded.

† Quinine, quinacrine, and chloroquine were assayed at concentrations of 0, 10, 25, 100, 500, and 1000  $\mu\text{g./ml.}$

‡ Trypsin was assayed at concentrations of 0, 93.8, 187.5, 375, 750, 1500, and 3000 u./ml.; 1  $\mu\text{g.}$  equals 2 units.

ship between the amount of penicillin inactivated to form penicilloic acid and the amount of carbon dioxide released from sodium bicarbonate as a result of the acid production. The activity curves in both figures represent best fitted lines for a relatively large number of values. For each compound, at least three replicate experiments were run at 6 to 10 inhibitor concentration levels.

It can be seen from figure 1 that trypsin and chymotrypsin are the most active penicillinase inhibitors at all inhibitor concentrations tested (10 to 1000  $\mu\text{g./ml.}$ ). The activity of quinacrine closely approximates that of chymotrypsin, after a minimum concentration of 25  $\mu\text{g./ml.}$  of quinacrine has been reached. Chloroquine activity is greater than that of quinine at concentrations up to 750  $\mu\text{g./ml.}$  Quinine shows no activity whatever at concentrations below 100  $\mu\text{g./ml.}$  At the 10  $\mu\text{g./ml.}$  level, only trypsin and chymotrypsin have any inhibitory effects: 41 and 31 per cent, respectively. At the 25, 100, and 1000  $\mu\text{g./ml.}$  levels, respectively, the various percentage inhibitions of penicillinase are: trypsin, 50, 62, 83; chymotrypsin, 37, 45, 58; quinacrine, 32, 42, 58; chloroquine, 4.6, 20, 46; and quinine, 0, 0, 48.

The penicillinase-inhibiting activities of the group of compounds compared in figure 2 are relatively slight. Only sulfathiazole and Xylocaine have any activity below the 200  $\mu\text{g./ml.}$  level. Above this inhibitor level, sulfanilic acid and sulfadiazine are fairly active, with maximum inhibitions of 19 and 14 per cent, respectively. D-penicillamine shows no inhibitor activity whatever in the concentrations tested (100 to 1000  $\mu\text{g./ml.}$ ).

To determine their relative inhibitory effectiveness against a higher concentration of penicillinase, trypsin, chloroquine, and quinine were mixed with four times the previous amount of the enzyme, i.e., 104 u. To assay this concentration of penicillinase, the iodometric method of penicillin measurement was used. Quinacrine inhibition could not be measured iodometrically, since this compound forms

an interfering colored solution. From figure 3 it can be seen that trypsin still exerts a pronounced effect, while quinine still is relatively ineffective. Chloroquine shows an appreciable proportionate increase in inhibitory power at the higher penicillinase concentration. The four to five points determining the curves graphed in figure 3 represent duplicate experiments. At the 100  $\mu\text{g./ml.}$  level, penicillinase percentage inhibition levels are: trypsin, 21; chloroquine, 17; and quinine, 0.

A single assay was done with trypsin at an 8 mg. penicillinase concentration (208 u.). The inhibitory powers of the various trypsin concentrations per ml. were approximately those at the 4 mg. (104 u.) level.

Bacteriological turbidity growth assays of the capacity of the antipenicillinase compounds to potentiate the growth inhibitory action of penicillin on the penicillin-resistant staphylococcal strain VCH 8 R are detailed in table I. These figures represent, in each case, the results of three replicate experiments. It was found that the compounds assayed (quinine, quinacrine, chloroquine and trypsin) have transitory augmenting effects, so that while comparatively low toxic concentrations are effective as penicillin-potentiators during the first eight hours of incubation, increasingly higher concentrations are required for inhibition of bacterial growth, as the time of incubation is lengthened. Quinacrine and trypsin are seen to be the most effective of the inhibitor compounds, as determined by their capacity to act at relatively low concentrations, so as to increase the antibacterial action of penicillin. After four to eight hours of incubation, relatively low concentrations of quinacrine (10 to 100  $\mu\text{g./ml.}$ ) or trypsin (93.8 to 375 u./ml.) potentiate the action of penicillin concentrations of 0.01 to 5 u./ml. However, the trypsin effect is lost after 24 to 48 hours of incubation, while the quinacrine effect is also found to decrease with increasing incubation time, so that increasingly higher concentrations of the compound are required for inhibition of staphylococcal growth. Quinine and chloroquine appear to be comparatively poor penicillin-potentiators, al-

TABLE II

*Sensitivity of Penicillinase-Producing and Non-Penicillinase-Producing Strains of Staphylococcus aureus to Penicillin\* and to Penicillin Plus Quinacrine (25  $\mu\text{g./ml.}$ )*

Strain number	Penicillin assay end points, u./ml.							
	Hours of incubation at 37 C.							
	With quinacrine				Without quinacrine (control)			
	12	16	20	24	12	16	20	24
VCH 1 R†	20	20	20	20	100	100	100	100
VCH 2 R	10	10	10-20	20	20	20	20	100
VCH 3 R	10	10	20	20	20	100	100	100
VCH 4 R	20	20	20	100	100	100	100	100
VCH 5 S‡	1	1	1	1	1	1	1	1
VCH 6 S	1	1	1	1	1	1	1	1
VCH 7 R	10	10	10	10	20	100	100	100
VCH 8 R	>100	>100	>100	>100	>100	>100	>100	>100
VCH 9 S	1	1	1	10	1	1	1	10
VCH 10 R	100	100	100	100	>100	>100	>100	>100
VCH 11 R	20	>100	>100	>100	>100	>100	>100	>100
VCH 12 R	100	100	100	>100	100	100	>100	>100
VCH 13 R	20	100	100	100	100	100	100	100
VCH 14 R	20	100	100	100	100	100	100	100
VCH 15 R	100	>100	>100	>100	>100	>100	>100	>100

\* Assay concentrations of penicillin were: 0, 0.1, 1, 10, 20, and 100 u./ml.

† R indicates a penicillinase-forming, penicillin-resistant strain.

‡ S indicates a non-penicillinase-forming, penicillin-sensitive strain.

TABLE III

Sensitivity of Penicillinase-Producing Strains of *Staphylococcus aureus* to Penicillin\*  
and to Penicillin Plus Trypsin (3000 u./ml.)

Strain number	Penicillin assay end points, u./ml.					
	Hours of incubation at 37 C.					
	With trypsin			Without trypsin (control)		
	17	24	48	17	24	48
VCH 1 R	10	10	10	10	10	10
VCH 7 R	10	10	10	10	10	100
VCH 8 R	10	10	100	100	> 100	> 100
VCH 10 R	10	10	10	100	100	> 100
VCH 11 R	10	10	10	100	100	> 100
VCH 12 R	10	10	> 100	10	> 100	> 100
VCH 14 R	10	10	100	100	100	100

\* Assay concentrations of penicillin were: 0, 0.1, 1, 10, and 100 u./ml.

though quinine, at a concentration of 25  $\mu\text{g.}/\text{ml.}$ , manifests temporary inhibition of growth of the test strain of bacteria when combined with 5 u./ml. of penicillin.

Table II compares the sensitivity of 12 penicillinase-producing and 3 nonpenicillinase-producing, penicillin-sensitive strains of *Staph. aureus* to penicillin. Both groups of organisms were then assayed against the same concentrations of penicillin, combined with 25  $\mu\text{g.}/\text{ml.}$  quinacrine. Ten of the 12 penicillin-resistant bacterial strains show increased sensitivity to the growth-inhibiting effect of penicillin when it is combined with quinacrine, as compared to their susceptibility to the antibiotic alone. However, only 6 strains continue to manifest comparative increased sensitivity to the drug combination after 16 or 20 hours of incubation, and only 5 strains still show a differential sensitivity after incubation for 24 hours. Quinacrine, in a concentration of 25  $\mu\text{g.}/\text{ml.}$  does not alter the penicillin assay end points of the 3 penicillin-sensitive strains of staphylococci.

The comparative sensitivities of 7 penicillinase-producing strains of *Staph. aureus* to penicillin and to penicillin plus 3000 u./ml. trypsin are presented in table III. After 48 hours of incubation, 4 of the bacterial strains still show increased sensitivity to penicillin when the antibiotic is combined with trypsin.

The efficacy of D-penicillamine (1000  $\mu\text{g.}/\text{ml.}$ ), Xylocaine (1000  $\mu\text{g.}/\text{ml.}$ ), and sodium sulfanilate (20,000  $\mu\text{g.}/\text{ml.}$ ) was tested also by bacteriological turbidity growth assays. D-penicillamine and Xylocaine were assayed on the penicillinase-producing strain VCH 8 R and the penicillin-sensitive strain VCH 5 S; sodium sulfanilate was assayed on the 3 penicillinase-producing strains: VCH 8 R, VCH 11 R and VCH 12 R. Sensitivities of the test strains of staphylococci to penicillin are exactly the same, in the presence or absence of these compounds, after 14, 18, 24, and 48 hours of incubation; neither D-penicillamine,  $\alpha$ -diethylamino-2,6-acetoxylidide nor sodium sulfanilate have any apparent effect as potentiators of the antibacterial action of penicillin.

#### DISCUSSION

*Staph. aureus* VCH 8 R, from which the penicillinase preparation was obtained, belongs to the virulent 42B/52A/80/81/VA4 phage type that has been found to be endemic in hospitals of the Miami area.<sup>33</sup> This organism produced a large amount of intracellular or firmly cell-bound penicillinase. In addition, large amounts of penicillinase were found in the sintered glass filtrate of the centrifuged 24 hour supernatant of the culture grown in brain-heart infusion broth (Difco).

Turbidimetric growth assays of 0.1 ml. of the filtered supernatant indicated that 500 u. penicillin were inactivated in 24 hour culture end points. Strain VCH 8 R also manifested an adaptive response to inclusion of penicillin in the growth medium. When 0.2 u. of penicillin was included in the culture media, 0.1 ml. of the filtered culture supernatant now inactivated 3500 u. of penicillin. As a result of these findings, consideration of the role of penicillinase interference with the chemotherapeutic action of penicillin should concern itself with the fact that at least certain virulent strains of staphylococci may elaborate large amounts of penicillinase that are not bound to the bacterial cell.

D-penicillamine, as one of the breakdown products of penicillin, has been investigated for its penicillinase-inhibiting action, because of its possible function as a competitor with penicillin for the site of enzyme adsorption. At all D-penicillamine levels up to 1000  $\mu\text{g./ml.}$ , no staphylococcal penicillinase inhibitory activity was found by direct enzyme assay, nor was the antibacterial action of penicillin potentiated. These findings are in contrast with those reported for D- and DL-penicillamine acting on penicillinase A and the penicillinase-producing organism, *Bacillus cereus*.<sup>20</sup>

Various sulfonamides have been reported to have penicillinase-inhibitory action.<sup>26</sup> In this study, sulfathiazole showed a slight inhibitory activity, while sulfadiazine showed a minimal effect in concentrations containing a minimum of 500  $\mu\text{g./ml.}$  These findings confirm the negative results reported for sulfadiazine in low concentrations.<sup>21</sup> In contrast, the presence of extremely high concentrations of sulfadiazine and/or sulfathiazole have been found to result in a marked protection of penicillin against penicillinase.<sup>25</sup> It was felt, however, that this protection was due to an adsorption of the enzyme on the sulfonamide particles; the degree of protection depended upon the amount of the suspended sulfonamide and its particle size.

The protection of penicillin by sodium sulfanilate, in concentrations from 1000 to 10,000  $\mu\text{g./ml.}$ , from destruction by a crude penicillinase preparation, was demonstrated using a penicillin-sensitive strain of *Staph. aureus* as the test organism.<sup>24</sup> It was also reported in the same study that penicillin blood serum levels could be prolonged by the administration of penicillin with sodium sulfanilate. In our studies of sulfanilate action on penicillinase, anti-enzyme activity began to be demonstrated at 200  $\mu\text{g./ml.}$  and reached a 19 per cent enzyme inactivation level at 1000  $\mu\text{g./ml.}$  At 20,000  $\mu\text{g./ml.}$  sulfanilate, however, the activity of penicillin was not potentiated against 3 penicillin-resistant strains of *Staph. aureus*. The apparent discrepancy between the reported findings with a penicillin-sensitive *Staphylococcus* and our findings with 3 penicillin-resistant strains may conceivably be due to the possible inability of sulfanilate to attack intracellular penicillinase.

$\alpha$ -Diethylamino-2,6-aceto-xylydide has been reported to inhibit extracellular penicillinase from *B. cereus* and intracellular penicillinase from *Staph. aureus*.<sup>28</sup> In Oxford cups containing penicillin, penicillinase, and  $\alpha$ -diethylamino-2,6-aceto-xylydide concentrations of the last as low as 1:20,000 (50  $\mu\text{g./ml.}$ ) inhibited either penicillinase so that penicillin-sensitive staphylococci seeded on assay plates were inhibited by the penicillin. However, in these same studies, turbidimetric growth assays of penicillin-resistant strains of staphylococci required  $\alpha$ -diethylamino-2,6-aceto-xylydide concentrations of 1000  $\mu\text{g./ml.}$  at penicillin concentrations of 1.25 to 2.5 u. to prevent growth. At lower  $\alpha$ -diethylamino-2,6-aceto-xylydide concentrations (166  $\mu\text{g./ml.}$ ), the lag phase of growth of a penicillin-resistant *Staphylococcus* in the presence of penicillin was prolonged for six hours. In our studies with

$\alpha$ -diethylamino-2,6-aceto-xylylidide, direct measurement of its inhibitory action on penicillinase showed that there was a 1 per cent inhibition at 10  $\mu\text{g.}/\text{ml.}$ , increasing to 10 per cent at 500  $\mu\text{g.}/\text{ml.}$ ; in our turbidimetric growth studies, this drug, in a concentration of 1000  $\mu\text{g.}/\text{ml.}$ , did not potentiate the action of penicillin when measured after 18 hours of incubation. In the previously reported growth assay,<sup>28</sup> no indication of the incubation period was given.

Quinine hydrochloride, in a concentration of 1000  $\mu\text{g.}/\text{ml.}$ , was reported to inhibit extra- and intracellular penicillinase preparations in Oxford cup assays.<sup>28</sup> In our studies, quinine was active in penicillinase inhibition at a minimal concentration of 100  $\mu\text{g.}/\text{ml.}$  The related compounds, chloroquine and quinacrine, were found to be more active than quinine, in that low concentrations inactivated appreciably more of the enzyme. The more active compound, quinacrine, gave a 32 per cent inhibition of penicillinase at the comparatively low concentration level of 25  $\mu\text{g.}/\text{ml.}$  In turbidimetric growth assays, the relative order of effectiveness of the inhibitor compounds was quinacrine, quinine, and then chloroquine. In these assays, the incubation period proved to be an important factor in determining the effectiveness of the three compounds as penicillin potentiators. The potentiating effects with relatively low concentrations of quinine and quinacrine were evident for as long as eight hours of incubation but not after 24 hours. It should be noted that the relative effectiveness of quinacrine, quinine, and chloroquine was found to parallel their relative potency as staphylococcal growth inhibitors in the absence of penicillin. These findings must be considered in evaluating our penicillin-potentiating results. The action of the quinine series of compounds may not have been on penicillinase alone, for these compounds have also been shown to have adverse effects on bacterial growth and enzyme systems other than penicillinase.<sup>34-38</sup>

The proteolytic enzymes, trypsin and chymotrypsin, were found to be the most active of the penicillinase-inhibiting compounds. The very low trypsin concentrations of 10 to 25  $\mu\text{g.}/\text{ml.}$  gave 41 to 50 per cent inhibition of the test concentration of penicillinase. With chymotrypsin, 10 to 25  $\mu\text{g.}/\text{ml.}$  levels gave 31 to 37 per cent inhibition. In turbidimetric growth assays, the incubation period, as with the quinine series, proved to be an important factor in determining the effectiveness of the inhibitor. Trypsin, at concentrations of 93.8 to 375 u./ml., potentiated penicillin, acting in concentrations of 0.1 to 5 u./ml. during the first four to eight hours of incubation. After 24 hours' incubation, considerably higher concentrations of trypsin were required to potentiate penicillin action.

The chemotherapeutic effectiveness of the penicillinase-inhibitor compounds considered is limited to the nontoxic attainable levels *in vivo*. In this regard, we need consider only the more effective penicillinase inhibitors: trypsin, chymotrypsin, quinacrine, chloroquine, and quinine. For trypsin and chymotrypsin we have not found reported attainable blood and tissue levels following therapy. However, in the apparently successful treatment with trypsin and chymotrypsin of trauma and inflammation,<sup>39</sup> respiratory infection,<sup>40</sup> thrombophlebitis,<sup>41</sup> and of edema reduction<sup>42, 43</sup> from 10 to 23 mg. per day were given to human beings without untoward results. Similarly, in experimental animal infections, up to 20 mg./Kg. of trypsin were given.<sup>44, 45</sup> With the quinine series of compounds, blood plasma levels of 10  $\mu\text{g.}/\text{ml.}$  are readily obtained.<sup>46</sup> However, with all three compounds much greater tissue concentrations (varying from 10 to 300 fold) are achieved.<sup>46</sup> The attainable plasma and tissue concentrations make feasible the chemotherapeutic trial in animals of these penicillinase inhibitors in concentrations shown to be effective *in vitro*.

## SUMMARY

The staphylococcal penicillinase inhibitory capacities of various compounds were assayed by manometric and iodometric methods. Trypsin, chymotrypsin, quina-crine, chloroquine and quinine were found to be effective inhibitors, in the order given.  $\alpha$ -Diethylamino-2,6-aceto-xylylidide, sulfathiazole, sodium sulfanilate, and sulfadiazine showed minimal inhibitory effects, while D-penicillamine was without effect in the concentrations tested. Turbidimetric growth assays of penicillin potentiation for penicillin-resistant, penicillinase-producing staphylococci indicated that trypsin, quinacrine, quinine, and chloroquine were effective.  $\alpha$ -Diethylamino-2,6-aceto-xylylidide, sodium sulfanilate, and D-penicillamine were found to be ineffective as penicillin potentiators. The more effective compounds are discussed as possible penicillin potentiators in the therapy of penicillin-resistant staphylococcal infections.

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## BIBLIOGRAPHY

1. FINLAND, M., AND HAIGHT, T. H.: Antibiotic resistance of pathogenic staphylococci; study of 500 strains isolated at Boston City Hospital from October, 1951 to February, 1952, A.M.A. Arch. Int. Med. 97:143, 1953.
2. ROGERS, D. E.: The current problem of staphylococcal infections, Ann. Int. Med. 45:748, 1956.
3. WHITE, A. C., AND KNIGHT, V.: Drug resistant staphylococci: Part I. Their distribution in hospital patients. Part II. Their role in human infection, South. M. J. 49:1173, 1956.
4. WISE, R. I.; CRANNY, C., AND SPINK, W. W.: Epidemiological studies on antibiotic-resistant strains of *Micrococcus pyogenes*, Am. J. Med. 20:176, 1956.
5. WELCH, H.: Principles and Practice of Antibiotic Therapy, New York, Medical Encyclopedia, Inc., 1954, chap. 3.
6. ABRAHAM, E. P., AND CHAIN, E.: Enzyme from bacteria able to destroy penicillin, Nature, London 146:837, 1940.
7. ANON.: Committee on Medical Research, Office of Scientific Research and Development, Washington, D. C., and Medical Research Council, London, Science 102:627, 1945.
8. SPINK, W. W., AND FERRIS, V.: Penicillin-resistant staphylococci; mechanisms involved in development of resistance, J. Clin. Investigation 26:379, 1947.
9. FLOREY, H. W.; CHAIN, E.; HEATLEY, N. G.; JENNINGS, M. A.; SAUNDERS, A. G.; ABRAHAM, E. P., AND FLOREY, M. E.: Antibiotics, vol. 2, New York and London, Oxford University Press, 1949.
10. WALLMARK, G.: Bacteriophage types, sensitivity to antibiotics, and penicillinase production of *Staphylococcus aureus* (*pyogenes*), Acta soc. med. Upsa., 59:209, 1953.
11. BARBER, M.: Drug resistance of staphylococci with special reference to penicillinase production. In: Ciba Foundation Symposium on Drug Resistance in Micro-Organisms: Mechanisms of Development, Boston, Little, Brown and Co., 1957, pp. 262-274.
12. KIRBY, W. M. M.: Properties of penicillin inactivator extracted from penicillin-resistant staphylococci, J. Clin. Investigation 24:170, 1945.
13. HOUSEWRIGHT, R. D., AND HENRY, R. J.: Studies on penicillinase. III. The effect of anti-penicillinase on penicillin-resistant organisms, J. Bact. 53:241, 1947.
14. GILSON, B. ST. C., AND PARKER, R. F.: Staphylococcal penicillinase; characteristics of enzyme and its distribution, J. Bact. 55:801, 1948.

15. CZEKALOWSKI, J. W.: Penicillin inactivators of bacterial origin, *J. Path. & Bact.* 62:85, 1950.
16. GERONIMUS, L. H., AND COHEN, S.: Induction of staphylococcal penicillinase, *J. Bact.* 73:28, 1957.
17. GOTS, J. S.: Production of extracellular penicillin-inactivating substances associated with penicillin resistance in *Staphylococcus aureus*, *Proc. Soc. Exper. Biol. & Med.* 60:165, 1945.
18. LURIA, S. E.: Test for penicillin sensitivity and resistance in *Staphylococcus*, *Proc. Soc. Exper. Biol. & Med.* 61:46, 1946.
19. ERIKSEN, K. R., AND HANSEN, D.: Observations on extracellular nature of staphylococcal penicillinase, *Acta path. et microbiol. Scandinav.* 35:169, 1954.
20. BEHRENS, O. K., AND GARRISON, L.: Inhibitors for penicillinase, *Arch. Biochem.* 27:94, 1950.
21. HENRY, R. D., AND HOUSEWRIGHT, R. D.: Studies on penicillinase. II. Manometric method of assaying penicillinase and penicillin, kinetics of the penicillin-penicillinase reaction, and effects of inhibitors on penicillinase, *J. Biol. Chem.* 167:559, 1947.
22. PERLSTEIN, D., AND LIEBMANN, A. J.: In vitro protection of penicillin from inactivation by penicillinase, *Science* 102:174, 1945.
23. TACKING, R.: Immunization experiments with penicillinase, *Acta path. et microbiol. Scandinav.* 36:61, 1955.
24. REID, R. D.; FELTON, L. C., AND PITTROFF, M. A.: Prolongation of penicillin activity with penicillinase-inhibiting compounds, *Proc. Soc. Exper. Biol. & Med.* 63:438, 1946.
25. CRONHEIM, G.; BAIRD, M. E., AND DANNENBURG, W. N.: Studies with penicillinase in the presence of sulfonamides, *Arch. Biochem.* 23:394, 1949.
26. MEYER-ROHN, J.: Über Penicillinase und ihre Inhibitoren, *Hautarzt* 5:264, 1954.
27. KORTH, W.: Weitere Untersuchungen über die Wirkung von Trypsin auf bakterielle Toxine, Bakterienwachstum, Agglutinine und Penicillinase, *Arzn.-Forsch.* 4:388, 1954.
28. VÁCZI, L., AND URI, J.: Studies on the enzyme penicillinase, *Acta microb. Hung.* 2:167, 1954.
29. ANON.: Iodometric titration for penicillinase. Personal communication from Schen Labs Pharmaceuticals, 1958.
30. GOTS, J. S.: Detection of penicillinase-producing properties of microorganisms, *Science* 102:309, 1945.
31. HAIGHT, T. H., AND FINLAND, M.: Modified Gots test for penicillinase production, *Am. J. Clin. Path.* 22:806, 1952.
32. JANN, G. J.; STREITFELD, M. M., AND SALLE, A. J.: Fastness of *Micrococcus pyogenes* var. *aureus* to subtilin and to combinations of subtilin and streptomycin, *J. Bact.* 63:353, 1952.
33. SALLMAN, B.; STREITFELD, M. M., AND EHRENKRANTZ, J.: Miami, Florida Hospital Survey, 1958. Unpublished data.
34. LAWRENCE, C. A.: In vitro effects of quinine, atabrine and substituted compounds upon gram negative bacteria, *Proc. Soc. Exper. Biol. & Med.* 52:90, 1943.
35. JOHNSON, F. H., AND SCHNEYER, L.: The nature of the quinine inhibition of bacterial enzyme systems, *J. Bact.* 47:406, 1944.
36. JOHNSON, F. H., AND LEWIN, I.: The action of quinine on dehydrogenase systems of *Escherichia coli*, *J. Bact.* 48:260, 1944.
37. BAMBAS, L. L.: Dehydrogenase characteristics of the pneumococci, *J. Bact.* 50:629, 1945.
38. JOHNSON, F. H., AND LEWIN, I.: The action of quinine on the rate of growth and disinfection of bacteria, *J. Bact.* 50:121, 1945.
39. INNERFIELD, I.: Buccal trypsin in trauma and inflammation, *AM & CT* 3:245, 1956.
40. INNERFIELD, I.; SHINER, I. S., AND DUANY, E. V.: Buccal and intramuscular trypsin in respiratory tract infection, *J. Thoracic Surg.* 32:372, 1956.
41. KRYLE, L.; ARNOLDI, C., AND KUPPERMAN, H. S.: The therapeutic effectiveness of trypsin in the treatment of thrombophlebitis, *Ann. New York Acad. Sc.* 68:178, 1957.
42. LICHTMAN, A. C.: Clinical experience with trypsin, *Ann. New York Acad. Sc.* 68:196, 1957.
43. FULLGRABE, E. A.: Clinical experiences with chymotrypsin, *Ann. New York Acad. Sc.* 68:192, 1957.
44. MARTIN, G. J.: Anti-inflammatory effect of trypsin, *Ann. New York Acad. Sc.* 68:70, 1957.
45. HLADOVEC, J.; MANSFELD, V., AND HORAKOVA, Z.: Inhibitory action of trypsin and trypsin-inhibitors on experimental inflammation in rats, *Experientia* 14:146, 1958.
46. SOLLMANN, T.: A Manual of Pharmacology, Ed. 8, Philadelphia, W. B. Saunders Co., 1957.

# Bacitracin-Neomycin Detergent Spray: Effectiveness in the Treatment of Massive Experimental Wounds

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The development and standardization in this laboratory of an experimental wound preparation and the evaluation of various therapeutic regimens have been reported previously.<sup>1-3</sup> Wounds inflicted bilaterally on the hind legs of goats were lethal, with untreated animals surviving approximately 20 hours. However, the survival time could be extended by appropriate therapy, with whole blood to 36 hours, or with parenteral broad-spectrum antibiotics to 50 to 70 hours. Indefinite survival was obtained only by radical surgery accomplished within four to six hours after injury and supplemented with antibiotics and whole blood.<sup>2</sup> Adequate débridement required in every instance either high thigh amputation or hip disarticulation.

Experimental local wound therapy, including irrigation with tap water, sterile saline, hydrogen peroxide, and antibiotics, produced moderate prolongation of survival of the wounded animals. Local refrigeration and tourniquet application, alone or combined, and supplemented with blood and antibiotics, increased the survival time considerably.<sup>3</sup> From the results of these direct and indirect therapeutic means, it was concluded that the wound itself and factors present therein are determinants of the irreversibility of the shock that is characteristic of this preparation.

The presence in the wound of large numbers of bacteria was first reported by Oppenheimer et al,<sup>4</sup> who found that the gram-positive flora was suppressed by effective antibiotics. Knecht et al<sup>5</sup> observed the presence of clostridia in the blood stream of untreated animals at approximately 60 per cent of their elapsed survival time. More recent studies<sup>6-8</sup> have incriminated lecithinase-positive clostridia growing in the wound as significant specific determinants of survival in this preparation. Wise et al<sup>9</sup> have studied the relationship between survival time, on one hand, and both the clostridial infection and the reduction in circulating blood volume, on the other. This relationship has been clarified by the development of a quantitative "wound factor," which embodies both of these elements and which shows a statistically significant correlation with survival for the animals studied. A subsequent analysis of these same data (unpublished) by multiple regression indicates that these two components of the "wound factor" are of approximately the same order of importance.

The possible clinical application of these findings to the treatment of casualties en masse has been the impetus for the investigations reported herein. A highly practical approach to the treatment of large numbers of casualties under exigent circumstances would be the development of an effective means of local therapy that would permit the delay of more definitive wound treatment. It was with this approach in mind that the present study was undertaken.

The wound preparation (bilateral wounds) used in previous studies was thought to be too severe to permit graded assessment of the efficacy of varied treatment measures; therefore, a unilateral injury was employed throughout this study. It was the opinion of the authors that a single massive wound would more closely resemble the majority of large soft-tissue wounds received in battle or in the event of a nuclear explosion. Any beneficial effect from a therapeutic regimen would also be magnified.

Bacitracin and neomycin sulfate (Mycifradin sulfate\*) were used in this study because of their activity against the bacterial components of the wound flora in this preparation. Bacitracin has been reported to have low tissue toxicity and not to be inactivated in the wound.<sup>10,11</sup> However, prolonged neomycin therapy was reported to be nephrotoxic and ototoxic.<sup>12</sup> This evidence, in the opinion of the authors, did not rule out the use of neomycin if administration was limited to a single application. Systemic penicillin has already established itself as an effective aid to wound therapy in this preparation and in clinical use.

#### EXPERIMENTAL STUDIES

*Wound Preparation and Therapeutic Regimens.* Texas Angora goats (castrated), weighing 35 to 50 Kg., were injured as described by Ochsner et al<sup>1</sup> on one hind extremity only, in contrast to the bilateral wounds. Tetryl, a high explosive, was detonated on a predetermined point on the right hind leg, producing a massive soft-tissue injury. The femur was invariably comminuted, and the major vessels were usually severed. During the wounding process, animals were under pentobarbital sodium anesthesia (10 to 12 mg./Kg.). Hemostasis was accomplished immediately, local medication applied, and the wound loosely packed with a clean but not sterile gauze dressing. To facilitate application of spray therapy, skin flaps and damaged muscle masses were raised during the spraying process. The animals were maintained in a restrained prone position on special carts for the duration of their survival. Food and water were offered to the animals several times a day throughout their survival.

Six groups of wounded animals were observed in these survival studies: Group 1: Wounded animals receiving local saline detergent spray only (12 animals). Group 2: Wounded animals receiving no local or systemic therapy (6 animals). Group 3: Wounded animals receiving local saline detergent spray in conjunction with intramuscular penicillin (12 animals). Group 4: Wounded animals receiving local bacitracin-neomycin detergent spray (12 animals). Group 5: Wounded animals receiving local bacitracin-neomycin detergent spray in conjunction with intramuscular penicillin (16 animals). Group 6: Wounded animals receiving local saline detergent spray, intramuscular penicillin, and intramuscular bacitracin-neomycin (16 animals).

With the exception of group 2, each experiment performed on a given day involved a simultaneous comparison of animals from each group.

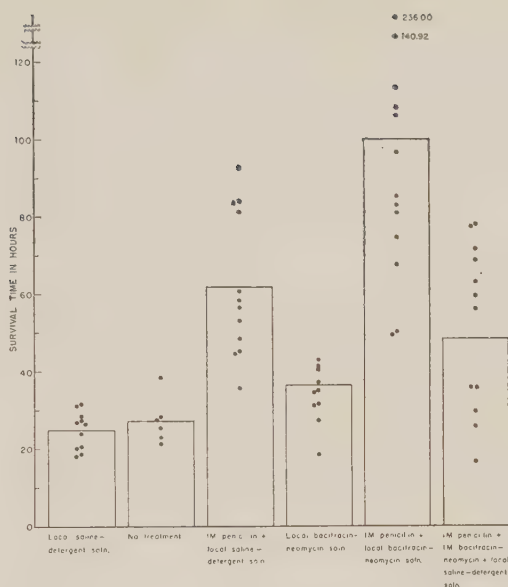
Animals treated with penicillin received a regimen of 1,000,000 units of crystalline and 300,000 units of procaine penicillin intramuscularly one hour prior to wounding, immediately postinjury, and every eight hours thereafter. Twenty-five thousand units of bacitracin and 250 mg. of neomycin were administered directly into the wound as an aerosol in 25 ml. of saline detergent solution (0.002 per cent di-octyl sodium sulfosuccinate in sterile normal saline) or intramuscularly in 5 ml. of saline. A standard atomizer under 10 pounds of direct pressure (nitrogen tank with regulator) was employed to spray the 25 ml. of antibiotic solutions or 25 ml. of the saline detergent solution without the antibiotic.

*Bacterial Estimations.* Serial samples were taken of the wound exudates at 9, 16, 25, 36, and 49 hours after injury. Quantitative estimations of the various types of organisms present were made on four selective or differential media: blood-azide—

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\* The trade name of The Upjohn Co. for neomycin sulfate is Mycifradin sulfate.

FIG. 1. Survival times of injured animals receiving the various therapeutic regimens. The top of the bar represents the mean for the group and the dots represent survival times of individual animals.



egg yolk agar (lecithinase-positive anaerobes), MacConkey's agar (gram-negative organisms), mannitol salt agar (staphylococci), and SF agar (fecal streptococci).

The method employed for estimating the count of the various organisms was that reported by Lindsey.<sup>13</sup> A standard dairy loop, which delivers 0.01 ml., was used to streak the surface of the various media in standard Petri dishes. Samples of the exudates were taken with standard cotton tip swabs, which delivered approximately 0.1 ml. of material. The swabs were rinsed in 0.9 ml. of cold sterile Trypticase soy broth and used as the initial dilution ( $10^{-1}$ ). The loop was then used to transfer 0.01 ml. ( $10^{-3}$  dilution) to the surface of the agar. Systematic streaking of the surface yielded subsequent dilutions up to  $10^{-8}$ .

## RESULTS

The survival time of each animal and the mean survival of each group are recorded in figure 1. The mean survival time and the standard error of the mean of each group are presented in order of decreasing efficacy in table I. It was observed that local bacitracin-neomycin therapy in conjunction with systemic penicillin was most effective in prolonging survival, and when the two groups were compared, the difference was statistically significant ( $p < 0.02$ ). There was no statistically significant difference between the survival times of animals receiving systemic penicillin alone and those receiving systemic penicillin and intramuscular bacitracin-neomycin. Local bacitracin-neomycin therapy alone significantly increased the survival time of the animals as compared with the control group ( $p < 0.05$ ).

The original protocol called for 12 animals in each group except group 2. For the statistical analysis 1 animal was dropped from group 1 and 1 from group 4 because an inadvertent variation in feeding the day before injury caused the animals to vomit and strangle several hours after wounding. Two animals in group 5 and 2 in group 6, all of which lived more than 300 hours, were found to have maggots in their wounds at the time of death. An additional 4 animals were added to each of the last two groups in an attempt to determine whether the maggots were responsible for, or the result of, the prolonged survival. Maggots were observed in 2 of these 8

TABLE I

*Survival Data of Wounded Animals According to Treatment*

Group no.	Treatment	No. of animals	Mean survival time, hours	Standard error of mean
5	Intramuscular penicillin plus local bacitracin-neomycin detergent solution	13	100.47	13.35
( $p < 0.02$ )*				
3	†Intramuscular penicillin plus local saline detergent solution	12	61.97	5.39
6	Intramuscular penicillin plus intramuscular bacitracin-neomycin plus local saline detergent solution	12	51.82	6.33
( $p < 0.05$ )*				
4	Local bacitracin-neomycin detergent solution	11	36.41	3.06
( $p < 0.05$ )*				
2	No treatment	6	27.30	2.48
1	†Local saline detergent solution	11	24.92	1.52

\* Probability that the difference between successive groups is due to chance alone.

† No statistically significant difference between successive groups.

animals approximately 70 hours after injury, indicating the possibility that the 2 markedly prolonged survivals may have been due to the beneficial effects of maggot débridement. It was therefore considered justifiable to drop all animals with maggots from the study. Maggots were observed in the wounds of 3 other animals, 1 animal in group 5 and 2 in group 6, 70 to 80 hours after injury; therefore they were sacrificed and dropped from the study.

Bacterial growth curves are presented in figure 2 and show the effect of various antibiotics and combinations thereof on the flora of the wound. Again the beneficial effects of bacitracin and neomycin are apparent, with the local treatment in conjunction with penicillin having the most marked suppressing effect on the wound flora. It should be noted that the 49 hour count recorded on the growth curves for

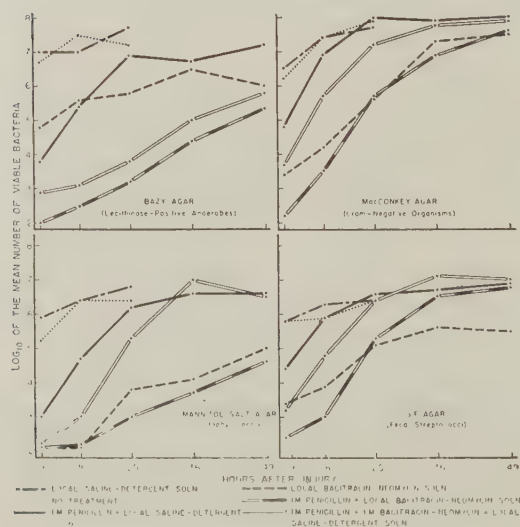


FIG. 2. Growth curves of the various organisms found in the exudates of the experimental wounds of the six groups of animals studied.

group 4 (local bacitracin-neomycin treatment) represents one determination from 1 animal.

The application of a saline detergent solution alone to the wound had little effect on survival (fig. 1) and wound flora (fig. 2). There was no statistical difference between group 1 and group 2 ( $p < 0.4$ ), as shown in table I.

#### DISCUSSION

The efficacy of the local application of bacitracin-neomycin to the wound in this experimental shock preparation was marked. Local therapy when employed alone or in conjunction with systemic penicillin was effective in suppressing the bacterial flora and, hence, in prolonging survival. This effect was most prominent when local bacitracin-neomycin and intramuscular penicillin therapy were combined.

Bacterial growth curves of the lecithinase-positive organisms, gram-negative organisms, staphylococci, and fecal streptococci demonstrate the action of local antibiotic therapy on the wound flora. When the local therapy was applied alone, the polymicrobial wound flora was reduced considerably, with a marked effect exhibited on the staphylococci and fecal streptococci. Combined intramuscular penicillin and local bacitracin-neomycin therapy dramatically reduced the lecithinase-positive organisms and the staphylococci counts. Little effect was noted on fecal streptococci. An initial suppression of the gram-negative organisms and the fecal streptococci was observed with all therapeutic regimens, including intramuscular bacitracin-neomycin and/or intramuscular penicillin. The control of the lecithinase-positive organisms (presumably *Clostridium perfringens*<sup>6</sup>) by this combined therapeutic regimen reduces the effect of a factor that is a definite detriment to the survival of animals in this wound shock preparation.

Local application of bacitracin-neomycin solutions by means of a spray has proved to be both practical and efficient in dispersing the antibiotic into the inaccessible areas of the wound. Di-octyl sodium sulfosuccinate, a wetting agent, was incorporated into the preparation to disperse the antibiotics into the frayed edges of the damaged muscles. The effectiveness of the detergent, however, was not evaluated in this study. Local detergent penicillin therapy was employed by Mitra and Grace<sup>14</sup> for the treatment of chronic osteomyelitis lesions with favorable results. Di-octyl sodium sulfosuccinate was the detergent employed in this investigation.

In previous reports of local chemotherapy, antibacterial agents were administered as a powder, as an ointment, or in solution either by irrigation or by saturating sterile gauze. The disadvantages of these various methods of application are quite apparent, especially if the situation requires the handling of a large number of casualties by inexperienced personnel, with inadequate facilities. •

Local application of bacitracin and neomycin has been shown to suppress the polymicrobial flora of the wound, suggesting possible clinical implications of this local therapeutic preparation in controlling surgical infections. It should be noted that the organisms most influenced by the local therapy were those normally sensitive to bacitracin and penicillin. By increasing the concentration of the antibiotics and/or repeating the application several times, a more prolonged action and even indefinite survival might possibly be obtained.

A "bacterial factor" in experimental shock preparations has been accepted, but the exact site or sites of bacterial action has been the subject of considerable controversy. Oppenheimer et al<sup>4</sup> and Lindsey et al<sup>6</sup> have established the presence of a

polymicrobial flora in the wound of this experimental shock preparation. Their studies and the work of Noyes et al<sup>8</sup> and Wise et al<sup>9</sup> have incriminated the clostridia present in the wound as significant determinants of the survival of the animals.

The efficacy of bacitracin-neomycin administered directly into the wound and the ineffectiveness of the same concentrations of the two antibiotics given intramuscularly confirm the conclusion that the wound itself is the site of the "bacterial factor" in this preparation.

Local chemotherapy of surgical infections, including war wounds, has been investigated employing the majority of the antibacterial agents available. With the advent of penicillin, numerous studies were undertaken to evaluate the effectiveness of the antibiotic in controlling surgical infections. Unlike the "medical" infections, surgical infections are usually limited to a particular area of damaged tissue. Parenteral penicillin in conjunction with local penicillin and/or local sulfonamides was employed with some success during World War II and the Korean conflict.<sup>15-22</sup>

With the efficient medical care available today, even under the hazard of war, as demonstrated in Korea, the incidence of gas gangrene infections is extremely low.<sup>23</sup> However, in the event of a nuclear disaster, this highly organized medical attention may be rendered ineffective and casualties may be without adequate medical treatment for hours or even days. Under these conditions wound infections could become a critical problem. It was for such situations that the local chemotherapeutic preparation reported here was developed. With it, an untrained assistant under minimum supervision can efficiently administer the treatment, which could possibly mean the difference between life and death of an injured person.

The technique of application and the composition of the detergent antibiotic solution employed in this limited series are not intended to be the ultimate in local chemotherapy of wounds, but are presented as a possible clinical approach to this yet unsolved problem of the care of casualties en masse. The optimum composition of the spray is a subject for further study. When this composition is decided, packaging of the solution in an integral, expendable sprayer package is quite a simple matter.

#### SUMMARY

1. Local bacitracin-neomycin detergent spray therapy in conjunction with intramuscular penicillin suppressed all the major components of the polymicrobial flora of the wound and significantly prolonged survival in this preparation.

2. The efficacy of local bacitracin-neomycin detergent spray and the ineffectiveness of intramuscular bacitracin-neomycin therapy definitely points to the wound as the site of detrimental bacterial action in this preparation.

3. The effectiveness of a sprayed detergent solution in dispersing antibiotics into the inaccessible areas of the wound indicates the possible clinical application of this technique.

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# BIBLIOGRAPHY

1. OCHSNER, E. W. A., JR.; JACOB, S. W., AND MANSBERGER, A. R., JR.: A new preparation for the study of experimental shock from massive wounds. I. Standardization, *Surgery* 43:703, 1958.
2. MANSBERGER, A. R., JR.; OCHSNER, E. W. A., JR.; JACOB, S.; OPPENHEIMER, J. H., AND GILLETTE, R. W.: A new preparation for the study of experimental shock from massive wounds. II. Evaluation of various therapeutic regimens with special reference to the role of antibiotics, fluid replacement, and débridement, *Surgery* 43:708, 1958.
3. MANSBERGER, A. R., JR.; OPPENHEIMER, J. H., AND GILLETTE, R. W.: A new preparation for the study of experimental shock from massive wounds. III. The effect of local wound treatment on survival time, *Surgery* 43:721, 1958.
4. OPPENHEIMER, J. H.; KNECHT, A. T.; MANSBERGER, A. R., JR.; GILLETTE, R. W., AND PON, R. S.: A new preparation for the study of experimental shock from massive wounds. VI. The effect of penicillin and streptomycin on the quantitative bacteriological flora of the wound, *Surgery* 43:730, 1958.
5. KNECHT, A. T., JR.; WISE, H. M., JR., AND BRINKLEY, F. B.: A new preparation for the study of experimental shock from massive wounds. VII. The occurrence of a clostridial bacteremia. *Surgery*. In press.
6. LINDSEY, D.; WISE, H. M.; KNECHT, A. T., AND NOYES, H. E.: The role of clostridia in mortality following an experimental wound in the goat. I. Quantitative bacteriology, *Surgery*. In press.
7. LINDSEY, D.; WISE, H. M.; KNECHT, A. T., AND NOYES, H. E.: The role of clostridia in mortality following an experimental wound in the goat. III. Effect of clostridial antitoxin in prolonging survival. *Surgery*. In press.
8. NOYES, H. E.; SHERMAN, R.; WISE, H. M.; KNECHT, A. T., AND LINDSEY, D.: The role of clostridia in mortality following an experimental wound in the goat. II. Toxicity of the wound exudate, *Surgery*. In press.
9. WISE, H. M.; LINDSEY, D., AND KNECHT, A. T.: Observations of the cardiovascular system in experimental shock. II. The nature of the "wound factor." To be published.
10. MELENEY, F. L.; LENGACRE, A. B.; ALTEMEIER, W. A.; REISNER, E. H.; PULASKI, E. J., AND ZINTEL, H. A.: Efficacy and safety of intramuscular administration of bacitracin in various types of surgical and certain medical infections. *Surg., Gynec. & Obst.* 89:657, 1949.
11. MELENEY, F. L.; JOHNSON, B. A., AND TENG, P.: Further experiences with local and systemic bacitracin in the treatment of various surgical and neurosurgical infections and certain related medical infections, *Surg., Gynec. & Obst.* 94:401, 1952.
12. WAISBREN, B. A., AND SPINK, W. W.: A clinical appraisal of neomycin, *Ann. Int. Med.* 33:1099, 1950.
13. LINDSEY, D.: Quantitative bacteriological study of body fluids and exudates. Some simplified methods, *J. Lab. & Clin. Med.* To be published.
14. MITRA, R. N., AND GRACE, E. J.: Further studies on the treatment of chronic osteomyelitis with topical detergent antibiotic therapy. In: *Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 455.
15. JENSEN, N. K., AND NELSON, M. C.: Local sulfanilamide in compound fractures; experimental and clinical evaluation, *Surg., Gynec. & Obst.* 75:34, 1942.
16. FLOREY, H. W., AND CAIRNS, H.: The treatment of war wounds with penicillin, *Brit. M. J.* 2:755, 1943.
17. BANON, J. N., AND MANSFIELD, O. T.: The local application of penicillin in soft-tissue lesions, *Brit. M. J.* 1:521, 1944.
18. ACKMAN, D., AND SMITH, F.: Bacteriological and clinical observations of the local treatment of infections and fresh trauma with penicillin cream, *Canad. M. A. J.* 51:493, 1944.
19. THOMSON, S.: The bacteriological examination of wounds treated with penicillin, *Brit. J. Surg.* 32:129, 1944.
20. BENTLEY, F. H., AND THOMSON, S.: Control of infection in recent war wounds by surgery and local chemotherapy, *Brit. M. J.* 1:471, 1945.
21. GLEDHILL, W. C.: Local and parenteral penicillin therapy in the treatment of recent war wounds, *Australian and New Zealand J. Surg.* 16:91, 1946.
22. FENNEY, P. W., AND MILLER, J. M.: Local chemotherapy of surgical wounds, *U. S. Armed Forces M. J.* 4:1031, 1953.
23. LINDBERG, R. B.; WETZLER, T. F.; MARSHALL, J. D.; NEWTON, A.; STRAWITZ, J., AND HOWARD, J. M.: The bacterial flora of battle wounds at the time of primary débridement; a study of the Korean battle casualty, *Ann. Surg.* 141:369, 1955.

# Studies on the Epidemiology of Strains of *Candida* in Hospital Wards

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Infections caused by organisms that resist the action of the available antibiotic agents have become a major problem. With many species of bacteria, the most important source of such infections appears to be the reservoir of resistant organisms that accumulate in the hospitals. This accumulation is believed to be related to the use of the drugs and is much more important in the occurrence of resistant strains of some organisms, e.g., staphylococci, than of others, e.g., tubercle bacilli.

Infections caused by strains of *Candida albicans* have also occurred while antibiotics were being administered. Since there is no convenient agent that can be administered orally that has appreciable systemic action, it has been proposed that a poorly absorbed antifungal agent be administered by mouth when an antibiotic that has a wide range of antibacterial action is being used. There are two main potential limitations of this method of control. In the first place, infection of areas such as the pulmonary and urinary systems may be principally exogenous rather than autogenous, in which case the local antibiotic would have little effect. Second, resistant strains may develop or be spread extensively if a large proportion of the patients were treated with the antifungal agents. From the data available concerning the development of resistant strains of *Candida*,<sup>1</sup> it would seem that the mechanism with the greatest potential for the production of resistance would be the spread of species other than *C. albicans*.

The amphotericins are very active against strains of *Candida* and have been shown to be quite effective when given by mouth in the control of these organisms in the stool. However, the commercially available form is most effective against systemic infections when given intravenously. It would, therefore, be most unfortunate if extensive oral use were to prejudice the parenteral effectiveness by favoring the accumulation of resistant strains.

This study has been done to estimate the extent of exogenous spread of *Candida* and to investigate the frequency of resistant strains or the development of such strains as a result of therapy. The former objectives would give information concerning the potential for spread to other than the gastrointestinal portal of entry as well as the possible dissemination of a resistant strain should such be introduced to the ward. The second objective coupled with the knowledge of the latter potential should give an approximation of the chances of accumulation of a population of resistant strains in the hospital when oral prophylaxis is used routinely.

## METHOD

The protocol outlining the source and type of patients, their treatment, and the method of isolation of the strains of *Candida* has been presented in another paper.<sup>2</sup> In that paper the species identification of the isolates has also been reported. After isolation, the organisms prepared in pure culture were grown on liquid media and the minimal inhibitory concentrations (MIC) of three substances were deter-

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TABLE I

*Distribution of the MIC Values for Three Compounds of Strains of C. albicans*

Compound tested	Total number of strains	Distributions of the MIC, $\mu\text{g./ml.}$							
		0.30	0.25	0.20	0.15	0.10	0.05	0.025	0.013
Brilliant green	111	0	1	5	28	57	20	0	0
Crystal violet	105	0	0	6	0	5	53	36	5
Amphotericin	104	0	3	8	17	26	50	0	0

mined: brilliant green, crystal violet, and amphotericin. Successive concentrations at 0.05  $\mu\text{g./ml.}$  increments were used from 0.05 to 0.40  $\mu\text{g./ml.}$  Below 0.05, a serial twofold dilution was used until 0.0125  $\mu\text{g./ml.}$  was reached. The least concentration that would inhibit growth as determined macroscopically was used as the end point. In each instance the inoculum was an 18 hour culture diluted to contain approximately  $10^4$  organisms/ml. In order to estimate the reliability of the method, the reproducibility of the MIC of these compounds was determined on the same isolates at intervals of one to six months.

Three methods for estimating spread have been used. First, species identification has indicated that there were relatively few implantations of new strains in patients who did not have *Candida* initially, and only two substitutions of one species for another were documented.<sup>2</sup> The second method has been to compare the changes in MIC of serial isolates from the same patient to ascertain whether these were greater than would be expected from errors in the laboratory as determined by repeated determinations on the same isolates. Since the number of tube changes possible on serial isolates depended on the distribution of the MIC of the population as a whole, a comparison of the distribution of the MIC values with the number of tube changes indicated the degree of similarity of serial isolates to random distribution and therefore the extent of possible random spread of exogenous strains. The third method has been to study the distribution of isolates with unusual MIC values to ascertain if there was any evidence for a pattern of spread. In this paper, data pertinent to the last two methods are reported.

## RESULTS

Table I summarizes the distribution of the MIC values of the three compounds. It can be seen that in the case of brilliant green, there were relatively few isolates different from the mode. With crystal violet, the majority of the strains were similarly distributed, but there were six that were in a separate, more resistant group. The distribution of the MIC values of amphotericin was unusual, since there were

TABLE II

*Distribution of Number of Tube Changes of the MIC Values for 3 Compounds of Strains of C. albicans Isolated Serially from Each Patient*

Compound tested	Total number of patients	Number of tube changes in the MIC										
		First greater than second					No change	Second greater than first				
		5	4	3	2	1		1	2	3	4	5
Brilliant green	51	0	0	0	2	6	30	10	2	1	0	0
Crystal violet	49	0	1	0	0	8	29	7	0	1	2	1
Amphotericin	61	0	0	1	2	13	31	12	2	0	0	0

TABLE III

*Distribution of the Number of Tube Changes in the MIC for 3 Compounds When Determinations Were Repeated on Strains of C. albicans after 1 to 6 Months in the Deep Freeze*

Compound tested	Number of patients	Number of tube changes in MIC						
		First greater than second			No change	Second greater than first		
		3	2	1		1	2	3
Brilliant green	52	0	0	5	44	3	0	0
Crystal violet	51	0	0	2	48	1	0	0
Amphotericin	45	0	3	3	35	3	1	0

no determinations below  $0.05 \mu\text{g./ml.}$ , even though this was the most frequent class.

In table II it can be seen that on repeated isolations, the brilliant green MIC values were the same in 60 per cent and the variation had a three tube maximum. With crystal violet, approximately 60 per cent remained the same, but among those varying by more than one tube, the degree of difference was three to five tubes. About 50 per cent of the strains isolated serially had identical MIC of amphotericin, and those that varied did so by up to three tubes. On the other hand, table III illustrates that repeated determinations on the same isolate preserved in the deep freeze infrequently varied and then only by one tube with the dyes and two with the antibiotic.

When the distribution in table II was compared with that in table III, it was possible to determine whether the results on serial isolates can be explained by laboratory errors. That the distributions were statistically different can be shown by the chi square values: brilliant green, chi square equaled 11.5, degree of freedom 5,  $p < 0.05$ ; crystal violet, chi square 17.8, degree of freedom 9,  $p < 0.05$ ; amphotericin, chi square 10.2, degree of freedom 4,  $p < 0.05$ . With crystal violet, if the five isolates with three or more tube changes were treated as a separate group, the number of one tube deviations was significantly greater in the serially isolated group (chi square 12.4, degree of freedom 2,  $p < 0.01$ ). That the dispersion of MIC values of the isolates was not dependent on the laboratory error was indicated by the comparison of the distributions in tables I and III, equating the mode class of table I with the no change class of table III. Here the values for brilliant green were: chi square 18.7, degree of freedom 4,  $p < 0.01$ ; for crystal

TABLE IV

*Date of Isolation of Strains of C. albicans That Required a High MIC of Crystal Violet*

Period of isolation	Number of strains with MIC, $\mu\text{g./ml.}$ , of	
	0.01 or less	0.20
Prior to December	32	1
December	18	0
January 1-20	21	0
January 21-31	5	5*
February and later	23	0
Total	99	6

\* One of these was present in patient on admission January 21. All others were isolated from patients after this date: 2 patients with single isolates and 1 with two.

TABLE V  
*Spread of Strains That Required a High MIC ( $\mu\text{g./ml.}$ ) of Crystal Violet*

Date	Patient A	Patient B	Patient C	Patient D
Prior to January 20		Three 0.05*		
January 20			0.013 admission culture	
January 21	0.20 admission culture			
January 22		0.05		
January 23				
January 24	0.025			0.025 admission culture
January 25				
January 26			0.20	
January 27				0.20
January 28	0.025			
January 29		0.20		
January 30				
January 31	0.025			0.20
Drug treatment	Tetracycline, 1/21 to 2/3, amphotericin, 5 mg./Kg./day, 1/28 to 2/3	Chloramphenicol and erythromycin, 1/27 to 2/10	Tetracycline, 1/20 to 1/27	Tetracycline, 1/24 to 2/7

\* Cultures taken on December 27 and 30 and January 7.

violet: chi square 29.2, degree of freedom 5,  $p < 0.01$ ; and for amphotericin: chi square 14.0, degree of freedom 4,  $p < 0.01$ . However, a similar comparison of the distribution of the tube differences on the serial isolates (table II) with the MIC values in table I revealed no essential difference for brilliant green where chi square was 6.8, degree of freedom 5, and  $p$  about 25. For crystal violet there was a difference, with chi square of 20.1, degree of freedom 9,  $p < 0.02$ . Similarly, there was also a significant difference for the amphotericin MIC distributions, with a chi square of 22.0, degree of freedom of 6, and  $p < 0.01$ .

There were only six isolates that had an MIC pattern distinct enough to suggest that the use of the label would permit study of the spread of a strain. As seen in table IV, one of these was isolated in November and the rest about the same time, in January. The single early isolate was from a patient who had three prior isolates over a 10 day period with a crystal violet MIC of 0.05  $\mu\text{g./ml.}$  and one with 0.025  $\mu\text{g./ml.}$  The fifth isolate on November 30 had an MIC of 0.20  $\mu\text{g./ml.}$  The first four isolates had a brilliant green MIC of 0.10  $\mu\text{g./ml.}$ , and for amphotericin 0.10  $\mu\text{g./ml.}$ , whereas on the fifth isolate these MIC values were 0.20  $\mu\text{g./ml.}$  and 0.05  $\mu\text{g./ml.}$ , respectively. It seems likely that this was a new strain that was acquired while the patient was being treated with tetracycline and amphotericin, 2.5 mg./Kg./day. Table V indicates the relationship between the other isolates. Patient A seems to have introduced the strain but then to have lost it, whereas the other patients subsequently acquired it within the next week. The brilliant green and amphotericin MIC values of these strains as obtained from the various patients was usually the same (0.10  $\mu\text{g./ml.}$ ), with one exception of 0.15  $\mu\text{g./ml.}$  for the dye and 0.05  $\mu\text{g./ml.}$  for the antibiotic, except one that was 0.10  $\mu\text{g./ml.}$

#### DISCUSSION

The difficulties encountered in the identification of strains limit the ability to demonstrate the spread of organisms. In the case of the *Staphylococcus*, for instance, the ability to type strains by the use of bacteriophage greatly aided the

study of the origin and spread of antibiotic-resistant organisms. However, even before this, the possibility of spread had been discerned from careful study of the distribution of the MIC of various antibiotics. In this and the preceding<sup>2</sup> paper, an identification has been attempted by using chemical agents for species (sugars) and strains (dyes and antibiotics). The use of species identification was limited by the fact that there was a marked preponderance of *C. albicans* among the isolates, which made the expectation of implantation of other species than *C. albicans* unlikely. The fact that there were a few such changes indicated that there was some potential for spread. On the other hand, the fact that most of the patients who did not have *Candida* never acquired them indicated that the potential may not have been great. Another explanation of this low rate of acquisition of implants may have been explainable by a resistance to *Candida* rather than a failure to have been exposed. The data in this paper, which indicated that there was more variation of the MIC values for the three agents used among serial isolates than could be accounted for by laboratory error, may indicate considerable transmission of the organisms. It is felt that the estimate of laboratory error as given in table III was reliable, since the determinations were run at a long interval and the bacteriologists were not aware of the previous results when the second reading was made. Moreover, two workers performed duplicate determination on some of the organisms and were able to check their own and each other's results. This method of estimating the extent of spread was seriously limited, however, by the fact that the population of *Candida* studied was quite homogeneous in its response to the agents. In spite of this, the fact that with two of the agents the distribution of the tube changes of serial isolates was less than the range of MIC would indicate that the former was more homogeneous and that the same strain probably was isolated from the same patient serially rather frequently.

The study of the six strains whose crystal violet sensitivity set them apart from the rest of the organisms certainly suggested that intraward dissemination occurred. It is unlikely that laboratory error accounted for these aberrant isolates since the work was done on the same day with many of the others that were in the usual range, they were done on different days from one another, and they were repeated at different times. Moreover, in the case of the isolates from the patient in November, the shift of the brilliant green MIC was relatively large. In the case of the isolates obtained in January, the data in table V strongly suggested the introduction of the organism by patient A and rapid transmission to the other patients. Moreover, since there were only five other isolates in this time period, it is apparent that for a brief period it was spreading to a high percentage of patients on the ward.

These data suggested therefore that there was some spread of strains of *Candida* in the hospital situation but the methods used did not allow a quantification of the extent. This study suggested a fairly extensive phenomenon, whereas the study of acquisition of new species and changes in species suggested that it was limited. It is possible, however, that should antibiotic-resistant strains develop or be introduced, they could become predominant on a given ward. The uniform sensitivity of all strains, and particularly the fact that the amphotericin MIC did not increase in those strains recovered from patients who received the drug, were reassuring.

#### SUMMARY

1. The distribution of the MIC of brilliant green, crystal violet, and amphotericin for strains of *C. albicans* isolated serially from the stools of patients in a small

pertussis ward suggested that strains of this species were spread from patient to patient with some frequency.

2. The evidence of spread of a given strain from 1 patient to 3 others in a one week period helped confirm the potential for spread of one *C. albicans* strain to a patient with another.

3. Some implications of this spread were discussed.

#### BIBLIOGRAPHY

1. LITTMAN, M. L.; PISANO, M. A., AND LANCASTER, R. M.: Induced resistance of candida species to nystatin and amphotericin B. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 981.
2. LEPPER, M. H.; SPIES, H. W., AND RUBENIS, M.: Use of amphotericin to eliminate *Candida* from the stools of infants treated with tetracycline. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 672.

# Use of Amphotericin to Eliminate *Candida* from the Stools of Infants Treated with Tetracycline

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For several years it has been generally accepted that infections by strains of *Candida albicans* may complicate the course of patients being treated with antibacterial agents, particularly those that eradicate a large proportion of the "normal" flora. While the magnitude of this problem has not been clearly defined, it is reasonable that prophylactic antifungal treatment should be used in patients with a high predisposition for *Candida* infection, such as diabetic persons, when broad-spectrum therapy is used. There has been a succession of drugs proposed for such prophylactic purposes, one of the most successful of which was nystatin.

In 1955 Gold and co-workers<sup>1</sup> described the isolation of a new mixture of antifungal agents, amphotericin A and B, from a streptomycete. Vandeputte et al<sup>2</sup> established that these compounds had characteristics of conjugated tetraenes similar to those of nystatin. For this reason the drug has been tested and recommended for the control of *Candida* in the stool similar to the use of nystatin.<sup>3</sup>

As part of a study in which we have attempted to investigate the possibility of spread of *Candida* from patient to patient in the hospital ward, amphotericin was used selectively to encourage resistant strains of *Candida* if such were present. However, since the subjects were all infants, it became necessary to determine an effective dosage on a weight basis. In this paper the results of these studies are reported along with data indicating the prevalence of various strains of *Candida* and the frequency of their acquisition in the hospital.

## METHODS

All patients were admitted to the pertussis wards of the Municipal Contagious Disease Hospital in the 10 months from July, 1957, to May, 1958. Approximately 50 per cent of the children were infants less than 1 year of age, and the majority of the others were between 1 and 2 years old. The oldest subject was 5 years of age. All patients were treated with tetracycline, 50 mg./Kg. for two weeks. Stool cultures were obtained before treatment was begun, and on the fourth, seventh, eleventh, and fourteenth days. In those patients who received amphotericin, the drug was started in the prescribed dosage on the eighth day of tetracycline therapy and both drugs were continued for one week. An attempt was made to treat 20 patients with each of four dosage regimens of amphotericin A and B. However, because some patients proved not to have pertussis, the entire course of therapy was not completed, and the patients were dropped from the group. Three of the dosage levels studied were 2.5 mg./Kg., 5.0 mg./Kg., and 10 mg./Kg./24 hours in divided doses given every six hours. The fourth regimen was 50 mg. every six hours, which in every instance amounted to more than 10 mg./Kg.

Stool cultures were made on Sabouraud's maltose agar containing 40 units/ml.

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TABLE I  
*Fermentation Reactions Used for Identification of Candida Species*

<i>Candida</i> species	Maltose	Lactose	Dextrose	Sucrose
<i>C. albicans</i>	A/G	—	A/G	A $\pm$
<i>C. tropicalis</i>	A/G	—	A/G	A/G
<i>C. krusei</i>	—	—	A/G	—
<i>C. parakrusei</i>	—	—	A/G or A only	—

of streptomycin and 20 units/ml. of penicillin. In each patient undiluted stool was streaked for culture and 0.1 Gm. was diluted in 10 ml. of saline. One tenth ml. of this suspension and serial tenfold dilutions of it were also cultured on the same media. This allowed a determination of one logarithm change in concentration of the organisms. Species of *Candida* were identified by typical chlamydospore formation on Zein media and by fermentation studies with maltose, lactose, dextrose, and sucrose. Table I gives the criteria used for species identification in the study.

### RESULTS

The results as far as *C. albicans* was concerned are tabulated in table II. One hundred and eighty-nine patients were admitted to the study, among whom 57 (30 per cent) had *C. albicans* isolated from the initial stool cultures. In 59 patients the studies were incomplete and only the initial culture was tabulated. Fifty-nine other patients received tetracycline without amphotericin. Among these 41 whose initial cultures were negative, only 3 became positive. Three of the 18 patients who had *Candida* isolated from the initial stool specimen never had it isolated again. Only three quantitative studies were done among those patients in whom strains persisted. In 1 patient the number decreased by at least one logarithm, in 1 it increased, and in 1 there was no change. Among the 71 patients who received tetracycline for one week before amphotericin was started, only 1 of 48 (2.1 per cent) patients acquired *Candida* and all but 1 of the 23 patients with positive cultures retained the organism. Ten of the remaining 22 patients did not have a change in number of organisms, whereas in 7 there was a one logarithm or more increase and in 5 a decrease of similar magnitude. Thus the changes that occurred after two weeks of tetracycline administration were not very different from those that occurred in the first week.

Even though four dose levels of amphotericin were studied, it is convenient to consider the results in those patients who received 5 mg./Kg./day or less compared to those who received 10 mg./Kg./day or more. In the lower dosage groups, 1 among 20 patients with negative prior cultures became positive, whereas all 28 similar patients in the higher dosage group remained *Candida*-free. On the other hand, in the lower dosage group, in only 3 of the 13 patients with positive cultures did the organisms disappear, whereas stools of 9 of 10 patients who received the higher dosage became negative. This difference was significant by the chi square test (chi square = 10.29). In the patient with persistently positive cultures in the higher dosage group, the number of organisms decreased by more than one logarithm, whereas in the other group this occurred in 3 patients, but in another there was an increase and in 6 no change.

In table III the occurrence of other species of *Candida* is tabulated. Only those groups in which one of these strains was found are listed. There were too few iso-

TABLE II

*Effect of Tetracycline and Amphotericin plus Tetracycline on C. albicans in Stool*

Therapeutic group	Effect of tetracycline on culture						Effect of amphotericin in addition to tetracycline											
	Patients with initial culture		Fate of negative culture		Fate of positive culture		Fate of negative culture		Fate of positive culture		Fate of negative culture		Fate of positive culture					
			Remained negative	Became positive	Became negative	No. decreased			No. increased	No. change			Remained negative	Became positive	Became negative	No. decreased	No. increased	No. change
Negative	Positive																	
Tetracycline + 2.5 mg./Kg. amphotericin	11	4	10	1*	1	—	1	2	10	1	—	1	—	3				
Tetracycline + 5 mg./Kg. amphotericin	9	9	9	—	—	2	4	3	9	—	3	2	1	3				
Total low-level amphotericin	20	13	19	1	1	2	5	5	19	1	3	3	1	6				
Tetracycline + 10 mg./Kg. amphotericin	13	8	13	—	—	3	2	3	13	—	7	1	—	—				
Tetracycline + 200 mg./Kg. amphotericin	15	2	15	—	—	—	—	2	15	—	2	—	—	—				
Total high-level amphotericin	28	10	28	—	—	3	2	5	28	—	9	1	—	—				
Tetracycline only	41	18	38	3	3	1	1	13†										
Total results with tetracycline	89	41	85	4	4	6	8	23†										
Only one culture	43	16‡																
Total initial cultures	132	57‡																

\* Replaced *C. tropicalis*.

† Quantitative studies not done in 12 of the patients.

‡ One also had *C. tropicalis*.

TABLE III

*Effect of Tetracycline and Amphotericin Plus Tetracycline on Candida Species  
Other than C. albicans in Stool*

Therapeutic group	Number in group	Number with non- <i>albicans Candida</i>		Remained positive on tetracycline	Fate on amphotericin	
		<i>C. tropicalis</i>	Other		Remained positive	Became negative
Tetracycline + 2.5 mg./Kg. amphotericin	15	1	—	0*	1†	—
Tetracycline + 10 mg./Kg. amphotericin	21	2	—	2	—	2
Tetracycline + 200 mg. amphotericin	17	1	—	1‡	—	1§
Tetracycline only	59	3	1§	4	—	—
Initial culture only	59	9¶	—	—	—	—
Total	189	16¶	1#			

\* *C. tropicalis* replaced by *C. albicans*.

† *C. albicans* persisted.

‡ *C. tropicalis* replaced by *C. parakrusei*.

§ *C. parakrusei* disappeared.

|| *C. tropicalis* appeared in stool of 1 of the 56 negative patients.

¶ One mixed *C. tropicalis* and *C. albicans*.

# *C. krusei*.

lates to reach any statistically significant conclusion, but the trends parallel those with *C. albicans*.

It is interesting to note that in only 2 patients did one species of *Candida* replace another. In 1, *C. albicans* replaced *Candida tropicalis*, and in the other, *Candida parakrusei* replaced this same species.

#### DISCUSSION

In a previous study, Halde and co-workers<sup>3</sup> have shown that 2 among 15 adult patients had a one logarithm increase in the number of *Candida* in the stools when 800 mg. of amphotericin was added to tetracycline after this drug had been used alone, whereas when 200 mg. was used, 6 of 13 patients showed an increase, but only 3 were of one logarithm or greater magnitude. It is likely that the larger dosage averaged slightly greater than 10 mg./Kg./day, whereas the lower dosage was nearer 3 mg./Kg. The results reported in this paper in infants showed a similar trend, but the differences seemed to be more definite. If amphotericin were included with tetracycline in a single dosage form so that 2 Gm. of the latter would provide 800 mg. of the former, a dosage of tetracycline of 25 or 50 mg./Kg. in children would provide sufficient amphotericin. In the previous study,<sup>3</sup> as in this one, the reason for failure of the drug to eradicate the organism more completely, particularly in the lower dosage group, is not clear. As reported elsewhere,<sup>4</sup> the greatest minimal inhibitory concentration of the organism encountered was 0.25  $\mu$ g./ml., and among those that persisted, it was 0.15  $\mu$ g./ml. It seems likely that the failure is not caused by resistance of the organisms.

The possibility that drug failure may have been caused by implantation of more resistant species of *Candida* was entertained, since Littman et al<sup>5</sup> showed that in some of these strains moderate resistance can be induced. That this did not occur in these patients is seen by the infrequency of change of species of *Candida* in

each patient. Moreover the minimal inhibitory concentration of amphotericin for strains of the non-*albicans* species were of the same order as that for the *albicans* strains.

From previous reports it is apparent that the order of activity of amphotericin in the higher dosage employed was similar to that demonstrated for nystatin.

In this study the effect of tetracycline on the occurrence of *Candida* in the stool was quite low. This may have been caused by the fact that the patients who received amphotericin were treated with the broad-spectrum drug for only one week prior to that time. However, in 59 patients who received the latter drug for two weeks, there was no real difference in those treated for two weeks. It is possible that in the hospital environment there were sufficient strains of other organisms resistant to tetracycline so that these organisms implanted rapidly enough to furnish sufficient competition to suppress the *Candida*.

#### SUMMARY

1. Among 189 patients, 56 had *C. albicans* in the stool, 15 had *C. tropicalis*, 1 had both of these, and 1 had *Candida krusei*.

2. Forty-one patients with negative cultures received tetracycline (50 mg./Kg./day) for two weeks, and in 3 the cultures became positive for *C. albicans* and in 1 for *C. tropicalis*. Of 18 patients in whom *C. albicans* was present in initial culture, it disappeared while the patient was receiving tetracycline in 3. Three strains of *C. tropicalis* and one of *C. krusei* also persisted.

Another 48 patients with negative cultures received tetracycline for one week; 1 acquired a strain of *C. albicans* and 1 a strain of *C. tropicalis*. Among the remaining patients treated for one week, only 1 of 23 with positive cultures lost the *C. albicans*. Among 4 patients with *C. tropicalis*, 2 remained positive and in the other 2 there was a change in species.

3. Among 20 patients with negative cultures who were treated with 5 mg./Kg./day or less of amphotericin, *C. albicans* appeared in 1. The cultures in 28 similar patients treated with 10 mg./Kg./day or more remained negative. On the other hand, in the lower dosage group, only 3 among 13 patients lost the *Candida* strains, whereas 9 of the 10 high dosage patients did.

4. A few implications of these findings are discussed.

#### BIBLIOGRAPHY

1. GOLD, W.; STOUT, H. A.; PAGANO, J. F., AND DONOVICK, R.: Amphotericins A and B, antifungal antibiotics produced by a streptomycete. I. In vitro studies. In: Antibiotics Annual 1955-1956, New York, Medical Encyclopedia, Inc., 1956, p. 579.
2. VANDEPUTTE, J.; WACHTEL, J. L., AND STILLER, E. T.: Amphotericins A and B, antifungal antibiotics produced by a streptomycete. II. The isolation and properties of the crystalline amphotericins. In: Antibiotics Annual 1955-1956, New York, Medical Encyclopedia, Inc., 1956, p. 587.
3. HALDE, C.; WRIGHT, E. T.; POLLARD, W. H. II; NEWCOMER, V. D., AND STERNBERG, T. H.: The effect of amphotericin B upon the yeast flora of the gastrointestinal tract in man. In: Antibiotics Annual 1956-1957, New York, Medical Encyclopedia, Inc., 1957, p. 123.
4. LEPPER, M. H.; LOCKWOOD, J.; SPIES, H. W., AND RUBENIS, M.: Studies on the epidemiology of strains of *Candida* on hospital wards. In: Antibiotics Annual 1958-1959, New York, Medical Encyclopedia, Inc., 1959, p. 666.
5. LITTMAN, M. L.; PISANO, M. A., AND LANCASTER, R. M.: Induced resistance of candida species to nystatin and amphotericin B. Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 981.

# Kanamycin: Its Cerebrospinal Fluid Diffusion, Renal Clearance, and Comparison with Streptomycin

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Kanamycin is an antibiotic whose activity may be expected to be of considerable value in the treatment of staphylococcal infections caused by strains less responsive to some of the agents that have been widely used in the recent past. Kanamycin shares many of the characteristics of streptomycin and neomycin and, hence, gives promise of being useful in the multifaceted attack on tuberculosis. Sharing, as it does, many of the characteristics of streptomycin and neomycin, kanamycin has similar potentials of otic and renal toxicity. Although the subject of kanamycin was recently reviewed *in extenso*,<sup>1</sup> certain particulars regarding the drug were lacking, and hence the work reported here was undertaken.

## CEREBROSPINAL FLUID DIFFUSION

In previous studies the diffusion of various substances through the uninflamed human meninges has been investigated.<sup>2-4</sup> In general, it has been found that drugs diffuse poorly into the cerebrospinal fluid and that when they do, the diffusion is a function both of the height of the circulating concentration of the drug and of the property of diffusibility possessed by the particular reference substance. Accordingly, it seemed to be of interest to determine to what extent kanamycin did, or did not, diffuse through the meninges.

All patients admitted to the hospital receive a spinal tap as part of a diagnostic work-up, and, accordingly, it is possible by appropriate premedication, to study the diffusion of many reference substances. The preliminary tests indicated that kanamycin did not interfere with any of the usual laboratory procedures carried out on cerebrospinal fluid, and hence, a protocol was set up whereby kanamycin was administered intramuscularly at periods of 2, 4, 6, 12, and 24 hours prior to the performance of lumbar puncture. Arbitrarily, the dose of 1 Gm. was selected for all intramuscular injections, in the belief that this dose probably represented the maximum single amount that would be administered to patients receiving kanamycin therapy. Most of the injections were made into the deltoid muscle or the upper outer quadrant of the buttock. Apart from occasional local soreness, there were no significant observations of irritation resulting from these injections.

In table I are presented the results of kanamycin assay of simultaneously obtained serum and cerebrospinal fluid specimens taken from 28 patients. The serum concentrations of kanamycin at the periods observed conform to those observed by others<sup>1</sup> and to those reported herein. At the peak of the serum concentrations observed during the second and fourth hours after the administration of kanamycin, traces of the antibiotic were observed in the cerebrospinal fluid. A "trace" is defined as that minimal amount of the antibiotic that was detected at the lower limit of the sensitivity of the assay procedure. Inhibition of the test organism was observed, but an actual zone beyond the size of the assay cup was not observed. After the sixth hour, no kanamycin was detected in the cerebrospinal fluid.

On the basis of these 28 patients to whom 1 Gm. of kanamycin was administered intramuscularly, it can be stated that the antibiotic does not diffuse through

TABLE I  
*Diffusion of Kanamycin into Cerebrospinal Fluid After Single  
1.0 Gm. Intramuscular Dose*

Pt.	Sex	Age, yr.	2 hours		4 hours		6 hours		12 hours		24 hours	
			Serum	Cere- bro- spinal fluid	Serum	Cere- bro- spinal fluid	Serum	Cere- bro- spinal fluid	Serum	Cere- bro- spinal fluid	Serum	Cere- bro- spinal fluid
A.G.	M	15	37.5	Tr.								
J.K.	M	51	52.5	Tr.								
G.A.	M	62	N.S.	Tr.								
C.N.	M	30	24.3	Tr.								
C.D.	M	19	32.5	Tr.								
C.M.	F	42			25.5	Tr.						
M.B.	F	39			19.5	Tr.						
M.B.	F	52			24.0	Tr.						
C.H.	F	45			24.0	Tr.						
C.P.	F	19			31.5	Tr.						
E.K.	M	32					12.5	Und.				
J.K.	M	19					12.0	Und.				
G.I.	F	38					6.5	Und.				
M.S.	F	76					N.S.	Und.				
L.M.	F	46					10.0	Und.				
M.B.	F	46					N.S.	Und.				
R.S.	F	38					13.0	Und.				
A.D.	M	19							0.72	Und.		
J.C.	M	16							1.10	Und.		
J.F.	M	32							N.S.	Und.		
L.F.	M	53							0.84	Und.		
J.T.	M	17							0.92	Und.		
T.H.	M	58							1.14	Und.		
J.W.	M	39							N.S.	Und.		
E.D.	F	30									Und.	Und.
A.D.	F	45									Und.	Und.
C.P.	F	19									Und.	Und.
D.M.	F	55									Und.	Und.

Tr. = 0.6  $\mu$ g./ml., lower limit of assay. N.S. = insufficient specimen. Und. = undetectable or <0.6  $\mu$ g./ml.

the uninflamed meninges of man. This finding was to be expected in view of previous work of a similar sort with reference to other substances. It is fully anticipated that other studies will show that kanamycin is found in the cerebrospinal fluid of patients suffering from meningitis or inflammation of the meninges due to other causes. The observations recorded here are in contrast to the findings that have indicated diffusion of kanamycin into the cerebrospinal fluid of dogs.<sup>5</sup>

TABLE II  
*Diffusion of Kanamycin into Saliva after Single 0.5 Gm. Dose Intramuscularly\**

Patient	Sex	Wt., lb.	Hours after dose		
			1	3	5
W.M.	F	112	Und.†		
M.M.	F	145	Und.		
H.F.	F	138		Und.	
L.J.	F	147		Und.	
M.M.C.	F	125			Und.
H.F.	F	112			Und.

\* These results were duplicated after a second intramuscular dose of 0.5 Gm.

† Und. = <0.6  $\mu$ g./ml. which is limit of assay; hence, "undetectable."

TABLE III  
*Kanamycin and Streptomycin Plasma Concentrations ( $\mu\text{g.}/\text{ml.}$ )  
after Single 0.5 Gm. Intramuscular Doses*

Male patients	Age, yr.	Wt., lb.	1 hour		2 hours		3 hours		5 hours		7 hours	
			K*	S	K	S	K	S	K	S	K	S
R.M.	21	178	18.8	14.0	16.4	13.4	11.8	11.6	5.2	5.4	2.4	4.9
M.D.	26	162	21.0	24.0	16.8	12.8	12.8	14.3	4.1	6.9	N.S.	3.4
H.C.	49	122	20.0	24.4	19.5	17.0	18.0	10.0	7.2	6.8	6.0	6.4
W.H.	34	128	N.S.	16.6	N.S.	12.0	10.1	13.1	4.8	7.8	2.1	3.6
E.W.	19	160	11.0	30.0	17.4	16.0	10.4	17.0	5.4	5.8	3.3	5.5
R.W.	35	212	10.5	14.2	15.8	11.6	11.1	15.5	6.0	6.0	3.3	5.5
Average			16.2	20.5	17.1	13.8	12.4	13.6	5.5	6.5	3.7	4.9

\* K = kanamycin. S = streptomycin. NS = insufficient specimen.

#### SECRETION INTO SALIVA

As another parameter of the diffusion of kanamycin into the tissues of the human body, an attempt was made to discover whether or not the antibiotic could be found in saliva. Six patients were injected intramuscularly with 0.5 Gm. doses, and at one, three, and five hours thereafter, these same patients were asked to expectorate into sterile jars. Fifteen minute collections of saliva were made, and these samples were submitted for antibiotic assay. These same patients received a second intramuscular injection of kanamycin three days later, and repeat samples were obtained. In table II are recorded our observations, which indicated undetectable quantities of kanamycin in all the specimens assayed. On the basis of these limited data, it is suggested that kanamycin is not secreted in significant amounts into human saliva.

#### KANAMYCIN AND STREPTOMYCIN COMPARED

Since kanamycin shares some of the properties of streptomycin, and both agents are applied to the therapy of tuberculosis, it seemed pertinent to determine in the same patients what the plasma concentrations might be after a similar intramuscular dose. Six patients were selected on the basis of their having essentially normal hepatic, renal, and cardiac functions, and then on separate occasions, with four days intervening between injections, the patients received injections of kanamycin and streptomycin. A crossover pattern of study was followed whereby 3 patients on the first day received kanamycin and the other 3, streptomycin; on the second day of study, medications were reversed.

In table III the plasma concentrations that were observed have been recorded. With the exception of patient E. W. at one hour, there was good correspondence between the concentrations of the two antibiotics at all points of comparison. The individual data conformed to the impression that is gained from examination of the average figures. It is well known that streptomycin, dihydrostreptomycin, and neomycin are toxic for the eighth cranial nerve, and it has already been established that kanamycin shares in this potential of otic toxicity.<sup>1</sup> Already there have been attempts to explain this toxicity on the basis of the size of the daily dose, the total number of Gm. administered, and the duration of time over which the drug was administered. Although all these items are factors to be considered, it is far more likely that experience and study will reveal the fact that ototoxicity is more closely related to the height of the plasma concentrations attained, regardless of the total Gm. administered.

TABLE IV  
*Plasma Concentrations of Kanamycin and Penicillin after Intramuscular Injections  
into the Same Patients*

Patient	Age, yr.	Wt., lb.	Hours after injection					
			2		4		6	
			K, $\mu$ g./ml.	P, units/ml.	K	P	K	P
C.R.	45	110	9.5	4.3	12.7	2.3	7.3	1.2
L.G.	45	135	15.2	6.0	16.1	2.9	9.3	1.5
A.K.	48	140	19.5	5.0	14.5	1.4	13.0	0.48
E.D.	70	170	22.5	3.8	14.0	2.0	8.6	1.5
A.L.	54	160	17.7	6.5	16.8	2.6	15.0	1.5
Average			16.9	5.1	14.8	2.2	10.6	1.2

K = kanamycin, 500 mg. intramuscularly (lot #D8494 Bristol).

P = penicillin, 500,000 units intramuscularly (lot #8F39258 Squibb).

In a study of a rather small group of patients, but nevertheless patients who were studied definitively, it has been demonstrated that there is a greater correlation between the height of streptomycin concentrations and ototoxicity than to either the number of Gm. administered or the duration of therapy.<sup>6</sup>

As can be seen from table III, the plasma concentrations of streptomycin and kanamycin are comparable. Accordingly, any observations of eighth nerve damage observed after the use of kanamycin cannot be adequately explained on the basis of concentrations of the antibiotic that are different from those with streptomycin. The definition of toxicity will rest on further studies showing differences between kanamycin and streptomycin at the physiological level, or as we suspect, future studies will establish that ototoxicity is observed when excessively high plasma concentrations (greater than 40  $\mu$ g./ml.) are attained due to overdosage or the administration of an average dosage of kanamycin to patients with impairment of hepatic or renal function.

#### PLASMA CONCENTRATIONS OF KANAMYCIN AND PENICILLIN

From the foregoing comparison of kanamycin and streptomycin plasma concentrations in the same patients, it is suggested that the two antibiotics are eliminated from the blood stream by a more or less common mechanism. Practically all antibiotics, other than penicillin, are protein bound to a considerable extent and, hence, their clearance by the human kidney appears to be substantially below that of glomerular filtration rate, and the decline of their serum concentrations is considerably slower than that observed with penicillin. It occurred to us that by injecting the same patients with kanamycin and penicillin on different days, the rates at which the antibiotics disappeared from the blood could be observed. Any differences in the slope of the declining curves might be interpreted as a difference in renal excretion pattern. In table IV are the observed plasma concentrations of kanamycin and penicillin, after the intramuscular injection of 0.5 Gm. and 500,000 units, respectively. The slope of decline for kanamycin is significantly less steep than that for penicillin.

In previous work,<sup>7</sup> it has been established that penicillin is excreted at the rate of renal plasma flow and that penicillin is not only excreted by glomerular filtration, but by active tubular secretion. The less steep decline of kanamycin plasma concentrations implies that its rate of renal clearance is considerably less than that for penicillin.

TABLE V

*Noneffect of Probenecid on Plasma Kanamycin Concentrations (μg./ml.)*

Pt.	Age, yr.	Wt., lb.	Hours after kanamycin*							
			1		3		5		7	
			Without	With†	Without	With	Without	With	Without	With
W.M.	27	112	18.1	28.6	17.0	20.7	9.8	9.7	4.2	5.3
H.F.	42	138	28.6	46.0	15.0	17.7	6.3	6.2	4.0	5.0
M.M.C.L.	36	125	17.2	23.6	12.9	16.9	6.2	6.6	2.5	3.1
M.M.	43	145	25.6	18.0	24.1	18.5	13.8	11.8	7.5	7.5
L.J.	38	147	27.6	22.8	16.5	15.0	7.4	8.0	3.1	5.9
H.Fr.	47	110	40.6	32.2	27.0	23.3	11.3	12.8	7.5	5.5
Average			26.4	28.5	18.7	18.7	9.1	9.2	4.8	5.4

\* Kanamycin, 0.5 Gm. intramuscularly.

† Probenecid, 2.0 Gm. orally two hours prior to kanamycin injection.

## EFFECT OF PROBENECID ON KANAMYCIN PLASMA CONCENTRATIONS

Since it has been established that the renal tubular component of penicillin excretion can be reversibly inhibited by probenecid,<sup>8</sup> it was thought to be of interest to determine whether or not probenecid had an effect on kanamycin serum concentrations. In a crossover pattern of study, the same 6 patients were studied on two different occasions. The first day, 3 patients received 2 Gm. of oral probenecid, two hours prior to an intramuscular injection of 0.5 Gm. of kanamycin. Three other patients received only the intramuscular injection of kanamycin. Blood samples were drawn at one, three, five, and seven hours after the injections of the antibiotic, and submitted for microbiological assay. With the reversal of medication, the same study was carried out five days later. This lapse of time between phases of the study was dictated by the necessity of having probenecid cleared from the circulation. In table V it can be observed that the serum concentrations of kanamycin were almost identical in the two phases of study, and it is quite clear that probenecid has no influence on kanamycin serum concentrations. The implication of these findings is that kanamycin is not excreted by the renal tubules of man.

## RENAL CLEARANCE OF KANAMYCIN

The data in tables III, IV, and V offer indirect evidence that kanamycin is cleared at a rate slower than that for penicillin and at a rate approximately equal to that for streptomycin. The fact that probenecid, a compound that is known to inhibit the tubular secretion of a number of reference substances, exerts no effect on the excretion pattern of kanamycin indicates rather clearly that kanamycin is excreted by glomerular filtration. However, to establish this point, it seemed de-

TABLE VI

*Renal Clearance of Kanamycin in D.K., a 32 Year Old Woman, Weighing 122 lb.*

Period	Minute	Plasma concentration, μg./ml.	Urine concentration, μg./ml.	Total urinary recovery, mg.	Kanamycin clearance, ml./minute	Creatinine clearance, ml./minute	Kanamycin/ creatinine clearance ratio
I	16	52.5-52.0	100	50	81.5	93.0	0.88
II	15	52.0-62.5	110	55	70.2	104.0	0.68
III	15	38-52.5	140	70	81.5	98.2	0.83
IV	15	62.5-55.0	140	70	79.8	95.5	0.84

sirable to carry out a conventional clearance. A young woman, aged 32, with normal renal functions, as indicated by normal nonprotein nitrogen, creatinine, uric acid, normal ability to concentrate and dilute urine, and without formed elements in the urine, was selected for a conventional renal clearance study. As a priming dose, 0.5 Gm. of kanamycin was injected intramuscularly, and then one hour later, a continuous intravenous infusion of kanamycin was begun. One Gm. of kanamycin was diluted in 1000 ml. of 5 per cent glucose, and this solution was infused at a rate of 100 drops/minute. After a one hour period of equilibration, quantitative urinary collection was begun. Urine was collected during four 15 minute periods and blood samples were drawn at the beginning and end of each of these periods. All specimens of blood and urine were assayed for their content of both kanamycin and creatinine. Creatinine was regarded as an approximate measure of glomerular filtration rate in man.

In table IV are presented the particulars of this investigation, and it may be observed that the clearance rate for kanamycin in this patient was somewhat less than that for creatinine. It is therefore suggested that kanamycin is cleared by the human kidney at a rate approximating that of glomerular filtration. Additional patients are presently being studied, with the idea of determining what the renal clearance of kanamycin is in normal persons as well as in those whose hepatic and renal functions are compromised. This work will be the subject of another communication.

#### SUMMARY AND CONCLUSIONS

In 29 patients it has been shown that kanamycin does not diffuse through the uninflamed human meninges after an intramuscular dose of 1 Gm. The plasma concentrations of kanamycin and streptomycin have been found to be comparable after the intramuscular injection of the two antibiotics into the same patients. Kanamycin apparently is not secreted into human saliva, as judged by our experience in 6 patients after the intramuscular injection of 0.5 Gm. of kanamycin. The declining slope of plasma concentrations of penicillin and kanamycin were compared in the same persons. The steeper slope of penicillin, which is known to be excreted in man by both glomerular filtration and tubular secretion, suggests that kanamycin is cleared at a slower rate and by a different excretion pattern. Probenecid, which is known to inhibit reversibly the tubular secretion of penicillin and a number of other reference substances, has been demonstrated to have no enhancement effect on the plasma concentrations of kanamycin. This, too, serves as indirect evidence that kanamycin is not secreted by the human renal tubule. The renal clearance of kanamycin by conventional clearance technique has been shown to be slightly less than the glomerular filtration rate, as measured by creatinine clearance. Kanamycin is cleared at a rate of approximately 80 ml./min. and the kanamycin/creatinine ratio is approximately 0.8.

It is suggested on the basis of the foregoing data that if kanamycin is administered, knowingly or unknowingly, to patients who have impaired renal function, the plasma concentrations will almost certainly be higher than anticipated, and with these elevations, toxic manifestations and particularly ototoxicity can be expected. In this regard, kanamycin is in no way different from neomycin and streptomycin, and it is recommended that when any of these agents are being employed, care should be taken to individualize therapy, and to measure the serum content when facilities for such measurements are available.

The authors are indebted to Mr. Myron Shoemaker and Mr. Vincent Cassella for their technical assistance in performing the antibiotic assays.

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## BIBLIOGRAPHY

1. ANON.: Conference on the basic and clinical research of the new antibiotic, kanamycin, New York Acad. Sc. July 10–11, 1958.
2. BOGER, W. P., AND WILSON, W. W.: Rapid attainment of therapeutic penicillin concentrations in the cerebrospinal fluid, *Am. J. M. Sc.* 217:593–599, June, 1949.
3. GYLFE, J.; BAYNE, G. M., AND BOGER, W. P.: Penethamate (Neo-Penil): Its diffusion into the cerebrospinal fluid, *Antib. & Chemo.* 4:133–137, Feb. 1954.
4. BOGER, W. P.: A new long-acting antibacterial sulfonamide, 2,4-dimethoxy-6-sulfanilamido-1, 3-diazine: a comparative study. *In: Antibiotics Annual 1958–1959*, New York, Medical Encyclopedia, Inc., 1959, p. 48.
5. TISCH, D. E.; HUFTALEN, J. B., AND DICKISON, H. L.: Pharmacological studies with kanamycin, *Ann. New York Acad. Sc.* 76:44, 1958.
6. CARR, D. T.; BROWN, H. A.; HODGSON, C., AND HEILMAN, F. R.: Neurotoxic reactions to dihydrostreptomycin, *J.A.M.A.* 143:1223–1225, Aug., 1950.
7. BOGER, W. P.; MATTEUCCI, W. V.; SCHIMMEL, N. H., AND FLIPPIN, H. F.: Benemid: Its influence on PAS, penicillin and other antibiotics; Scientific exhibit presented at the Atlantic City Session, American Medical Association, June 11–15, 1951.
8. BOGER, W. P.; BEATTY, J. O.; PITTS, F. W., AND FLIPPIN, H. F.: The influence of a new benzoic acid derivative on the metabolism of para-aminosalicylic acid (PAS) and penicillin, *Ann. Int. Med.* 33:18–31, July, 1950.

# Renal Clearance of Kanamycin in Children

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Kanamycin, an antibiotic recently discovered by Umezawa in Japan,<sup>1</sup> is a welcome addition to the currently available antibiotics. This agent appears to be effective against staphylococci resistant to many of the antibiotics in common use.<sup>2</sup> Its activity against tubercle bacilli and enteric organisms suggests that it may be useful for infections due to these organisms.<sup>3</sup> Poor absorption on oral administration increases its usefulness in preoperative preparation for surgery involving the lower intestinal tract.

The rapid absorption and excretion<sup>4</sup> of this drug after parenteral administration indicate that studies of the mechanism of excretion are needed. Knowledge of the renal mechanisms involved may allow better control of excretion and thus permit the administration of smaller and less frequent doses with perhaps less toxicity.

## MATERIALS AND METHODS

The patients studied ranged in age from 6 through 12 years. All were hospitalized for diagnostic studies or were convalescent from various relatively mild illnesses. None had received an antibiotic within 48 hours prior to study. There was no history of prior renal disease in any patient, and urinalysis was within normal limits during the hospital stay.

After an 8 hour fasting period, all patients were given a 20 ml./Kg. water load by the oral route and a 10 mg./Kg. intramuscular injection of kanamycin sulfate.† During the next two hour period (to avoid sample collections at the time of most rapid rise and decline of kanamycin serum levels), all urine was measured and discarded and an equivalent amount of water was given by mouth. At the end of the two hour stabilizing period, serum was obtained for kanamycin and creatinine assay. During the next hour, urine samples were collected at 15 minute intervals and serum samples at 30 minute intervals. All urine volumes and time intervals were measured accurately. Samples were stored at -20 C. for subsequent creatinine determinations and at 5 C. for kanamycin assays. Kanamycin assays utilized the cup-plate technique using *Bacillus subtilis* as the test organism.‡ Creatinine determinations were performed using the method of Hare.<sup>5</sup> Clearance data were calculated by methods described by Smith.<sup>6</sup>

Dialysis was performed using cellulose dialyzing tubes containing normal human serum to which different concentrations of kanamycin had been added. These tubes were suspended in 500 ml. of a phosphate buffer solution at pH 7.4 at 37 C. for 24 hours. Control samples for possible drug deterioration over the 24 hour dialysis period were also studied.

## RESULTS

Since the proportion of kanamycin available for excretion depends on the extent

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\* John and Mary R. Markle Scholar.

† The trade name of Bristol Laboratories Inc. for kanamycin is Kantrex. The drug was supplied for this study by Bristol Laboratories Inc.

‡ We are indebted to Miss Kay Cassen, who performed these assays.

TABLE I  
*Dialysis of Kanamycin in Normal Serum*  
(pH 7.4 at 37 C)

Kanamycin concentration, $\mu\text{g./ml.}$	Time intervals	
	Initial	24 hours
20 $\mu\text{g.}$ in serum	18	<0.4
Phosphate buffer	0	<0.4
40 $\mu\text{g.}$ in serum	41	0.5
Phosphate buffer	0	<0.4
20 $\mu\text{g.}$ serum control	18	18.0
40 $\mu\text{g.}$ serum control	38	41.0

of serum binding, this factor must be examined first. Table I indicates that there is no appreciable serum binding under the test conditions of pH 7.4 using normal serum-kanamycin mixtures containing 18 and 41  $\mu\text{g./ml.}$ , respectively. The drug was completely stable during the test period at both 37 C. and 5 C.

The data regarding clearance in the 5 patients studied are summarized in table II. Although some variation occurred in the clearance of both kanamycin and creatinine, the ratio, C kanamycin/C creatinine is surprisingly stable, ranging from 1.18 to 1.56. The data show that kanamycin clearance is in excess of that to be expected if glomerular filtration alone were involved.

When urine volumes in excess of 13 ml./minute/sq. M. were observed, corresponding creatinine clearances were elevated but kanamycin clearances were unchanged. In these few instances, therefore, the ratio C kanamycin/C creatinine decreased, reaching unity or below on three occasions.

#### DISCUSSION

It seems clear from these data that a factor other than glomerular filtration must be operative in the renal excretion of kanamycin, since these clearances were consistently greater than those of creatinine. These kanamycin clearances do not approach the clearances of penicillin reported by Eagle and Newman,<sup>7</sup> however. These investigators noted clearances of penicillin from 550 to 900 ml./minute, and comparisons with para-aminohippuric acid clearance suggested that these figures approximated total renal plasma flow.

Data regarding other antibiotics indicate that some variation exists in tubular excretory activity. While chlortetracycline appears to be excreted by the glomerulus with little tubular activity,<sup>8</sup> tetracycline is excreted by the tubule in amounts ap-

TABLE II  
*Comparative Renal Clearance of Endogenous Creatinine and Kanamycin\**

Test subject	Median† clearance	ml./minute/1.73 sq. M.	Clearance ratio, kanamycin/creatinine
	Kanamycin	Creatinine	
H. H.	105	75	1.40
P. K.	117	84	1.40
A. K.	103	66	1.56
D. K.	75	49	1.54
W. W.	98	84	1.18

\* Clearances done simultaneously for both substances.

<sup>†</sup> Median represents mid-point of three or four clearance periods in each subject.

proximately equal to the quantity cleared by filtration,<sup>9</sup> a finding consistent with unpublished data from our laboratory. Tubular excretion of erythromycin also appears to be likely,<sup>10</sup> although the comparative roles of the glomerulus and the tubule are not clear from the data available.

Further studies are in progress to determine the influence of tubular blocking agents on renal excretion of kanamycin. This effect would not be expected to be large, since more than half the clearance of kanamycin appears to be consistent with glomerular filtration.

#### SUMMARY

Studies of simultaneous clearance of endogenous creatinine and kanamycin in children suggest that more than half the clearance is by glomerular filtration. Tubular excretion seems responsible for some of the renal loss of kanamycin. Tubular blocking agents, while worthy of exploration, do not appear to be a likely means of delaying renal excretion to any considerable extent.

#### BIBLIOGRAPHY

1. UMEZAWA, H.; UEDA, M.; MAEDA, K.; YAGASHITA, K.; KONDO, S.; OKAMI, Y.; UTAHARA, R.; OSATO, Y.; NITTA, K., AND TAKEUCHI, T.: Production and isolation of a new antibiotic, kanamycin, *J. Antib., Ser. A.* 10:181-188, 1957.
2. WELCH, H.; WRIGHT, W. W.; WEINSTEIN, H. I., AND STAFFA, A. W.: *In vitro* and pharmacological studies with kanamycin. *Ann. New York Acad. Sc.* 76:66, 1958.
3. HEWITT, W. L., AND FINEGOLD, S. M.: Laboratory studies with kanamycin, *Ann. New York Acad. Sc.* 76:122, 1958.
4. BERGER, S., AND WEHRLE, P.: Kanamycin serum levels in infants and children, *Ann. New York Acad. Sc.* 76:129, 1958.
5. HARE, R.: Endogenous creatinine in serum and urine. *Proc. Soc. Exper. Biol. & Med.* 74:148-151, 1950.
6. SMITH, H. W.: The kidney. Structure and function in health and disease. New York, Oxford Univ. Press, 1951, p. 42.
7. EAGLE, H., AND NEWMAN, E.: The renal clearance of penicillins F, G, K and X in rabbits and man. *J. Clin. Investigation* 26:903-918, 1947.
8. SIROTA, J. H., AND SALTZMAN, A.: The renal clearance and plasma protein binding of aureomycin in man. *J. Pharmacol. & Exper. Therap.* 100:210-218, 1950.
9. ENGELUND, A.; TERP, P., AND TROLLE-LASSEN, C.: Studies on renal excretion of tetracycline. *Acta pharmacol. et toxicol.* 12:227-232, 1956.
10. LEE, C.; ANDERSON, R. C., AND CHEN, K.: Renal clearance of erythromycin. *Proc. Soc. Exper. Biol. Med.*, 88:584-587, 1955.

# A Limited Study on the Use of Kanamycin in Patients with Fibrocystic Disease of the Pancreas

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Fibrocystic disease of the pancreas is a generalized disease involving chiefly the pancreas, the respiratory system, and the sweat glands, the pulmonary involvement being the most important. Its progression determines the fate of the patient. Prior to the antibiotic era, most of these patients succumbed to severe respiratory infections in early infancy. Intensive use of antibiotic therapy prolongs the life of these children, and many of them survive infancy and early childhood. The bacterial species isolated from such patients are commonly resistant to many antimicrobial agents; no single agent uniformly eliminates the bronchopulmonary infections in these patients. Large dosages of antibiotics are effective in improving the clinical condition of these patients and in reducing the pulmonary pathology. Thus new antibiotic agents deserve a trial in these patients.

Kanamycin sulfate, an antibiotic of relatively low toxicity, recently isolated from *Streptomyces kanamyceticus*, has been found to be effective against many organisms including gram-negative bacilli and *Micrococcus pyogenes* var. *aureus*. The latter species commonly cause severe bronchopulmonary infections in patients with fibrocystic disease of the pancreas. The present study was undertaken to determine the effectiveness of this agent in children with this disease.

## MATERIAL AND METHODS

A total of 13 patients with fibrocystic disease of the pancreas were hospitalized between January and August, 1958, for the treatment of acute exacerbations of chronic bronchopulmonary infections. All patients were treated with kanamycin administered intramuscularly at intervals of 12 hours and concurrently by aerosol inhalation. Kanamycin solution was placed in a Vaponefrin nebulizer and an aerosol mixture was produced by compressed air or oxygen. The mixture was inhaled from the nebulizer or from a face mask.

These patients received a total of 17 courses of kanamycin administered by the intramuscular route and 14 courses of concurrent aerosol inhalation.

The age and weight of these patients together with the dosage regimens and the duration of treatment are summarized in tables I and II.

Nasopharyngeal and/or bronchial secretions were obtained from all patients for culture and sensitivity tests prior to, and in most patients during, kanamycin therapy. Clinical progress was followed during treatment and bacteriological studies were repeated in 10 patients who underwent 14 courses of treatment.

Serum and bronchial secretions were obtained simultaneously for assay from 6 patients after the intramuscular administration of kanamycin and also from 4 patients after aerosol administration of kanamycin. Bronchial secretions were collected during bronchoscopic examinations.

## LABORATORY METHODS

Kanamycin sensitivity of bacterial pathogens was performed by the standard tube-dilution method.<sup>1</sup>

TABLE I  
Distribution of Patients According to Age and Weight

Age		Weight	
Years	Number	Kilograms	Number
2 - 5	3	10 - 15	5
6 - 10	8	16 - 20	5
More than 10	2	More than 20	3

The method used to determine the concentration of kanamycin in serum and bronchial secretions was a modification of the penicylinder agar diffusion technique using *Bacillus subtilis* as the test organism.<sup>2</sup>

#### RESULTS

Clinical response was measured by general improvement, decreased coughing spells, disappearance of râles in the chest, lowering of the temperature, and reduction of the leukocyte count. The response was considered good if three or four of these criteria were met, fair if the general condition improved but abnormal physical findings persisted, and poor if there was no improvement during the course of treatment. The results of the 17 courses of treatment given to the 13 patients are shown in table III.

Other agents including one or more of the following, neomycin, chloramphenicol, oleandomycin, and streptomycin, were also employed during two of the nine courses of treatment where good results were obtained, and during two of the eight courses where fair results were noted. In three of the latter eight, kanamycin was administered by the intramuscular and aerosol routes for an initial period of seven days. In that interval, cough decreased and general improvement occurred but the physical examination of the chest showed no change. Thereafter, 2 patients were treated with a combination of neomycin and chloramphenicol, whereas the remaining patient received neomycin by the intramuscular route and streptomycin by aerosol inhalation. However, the condition of these 3 patients remained stationary despite the change of antibiotic therapy. These patients were discharged from the hospital to continue treatment at home.

Four patients who responded satisfactorily to kanamycin during their hospitalization continued to receive kanamycin by aerosol inhalation at home. The longest duration of kanamycin aerosol therapy was two months.

Roentgenographic examination of the chest was performed before and after nine courses of kanamycin therapy in 9 patients. Three patients showed evidence of slight improvement in the inflammatory processes, whereas 6 patients showed no

TABLE II  
Dosage Regimen According to Duration of Treatment and Number of Courses of Therapy

Intramuscular			Aerosol		
mg./Kg./day	Duration, days	Number of courses	mg./dose, used 3 times daily	Duration, days	Number of courses
20-40	7-12	6	100-150	7-15	3
41-60	7-11	4	200	8-11	4
61-80	8-15	4	250	7-18	7
100	11	3			
Total		17			14

TABLE III  
Clinical Responses of Patients with Fibrocystic Disease of the Pancreas to  
Kanamycin Treatment

Result	No. of courses
Good	9
Fair	8
Poor	0

change. This observation is consistent with previous experiences with other antibiotics. The prolonged existence of the pulmonary infections in such patients precludes dramatic improvement.

Bacteriological studies were obtained, and a total of 29 nasopharyngeal cultures, one throat culture, and 22 bronchial cultures were performed on the 13 patients treated with kanamycin. The results of the cultures are summarized in table IV.

Inspection of these data shows that only one of 13 nasopharyngeal cultures obtained prior to treatment failed to yield significant pathogens, while 5 of 16 cultures obtained during treatment showed no pathogens. This difference is not considered significant. All bronchial cultures taken prior to and during treatment were persistently positive for the same pathogens. Coagulase-positive hemolytic *Staphylococcus aureus* was the predominant pathogen in 7 patients, *Pseudomonas aeruginosa* was the predominant pathogen in 4 patients, and a combination of hemolytic *Staph. aureus* and *Ps. aeruginosa* was present in 2 patients.

The results of kanamycin sensitivity tests performed by tube-dilution method are presented in table V. The majority of staphylococci were susceptible to 15.6  $\mu\text{g./ml.}$  or less of kanamycin. Only 2 of 23 strains were susceptible to 62.5  $\mu\text{g./ml.}$ , a concentration that can be achieved in the serum after the intramuscular administration of kanamycin. The data presented showed no evidence of increased resistance of staphylococci to kanamycin during the short period of observation. Most strains of *Ps. aeruginosa* were found to be resistant to kanamycin. Only 2 of 10 strains tested were susceptible to 31.3  $\mu\text{g./ml.}$

Kanamycin concentrations in serum and bronchial secretions were determined after the administration of kanamycin by intramuscular and aerosol routes. The results are shown in tables VI and VII. Although the number of specimens tested was small, the results indicated that the kanamycin concentrations in the bronchial secretions were considerably higher than in serum after aerosol inhalation. A single

TABLE IV  
Bacteriological Studies in Patients with Fibrocystic Disease of the Pancreas  
Treated with Kanamycin

	Relation to treatment	No. of cultures taken	No. positive for hemolytic <i>Staph. aureus</i>	No. positive for <i>Ps. aeruginosa</i>	No. positive for coliform organisms
Nasopharyngeal cultures	Prior	13*	10	2	1†
	During or after	17	5	6	1
Bronchial cultures	Prior	7	7	2‡	—
	During or after	14	10	5(1‡)	—

\* Includes one throat culture.

† This organism and hemolytic *Staph. aureus* were isolated from the same culture.

‡ In these three cultures *Ps. aeruginosa* was isolated together with hemolytic *Staph. aureus*.

TABLE V

*Kanamycin Sensitivity Test on Staphylococci and Ps. aeruginosa Isolated from Patients with Fibrocystic Disease of the Pancreas*

Microorganism	No. of strains tested	No. of strains sensitive to kanamycin, $\mu\text{g./ml.}$									
		0.25	0.5	0.1	2.0	3.9	7.8	15.6	31.3	62.5	125 or greater
Hemolytic <i>Staph. aureus</i>	23	1	1	1	—	—	4	10	4	2	—
<i>Ps. aeruginosa</i>	8	—	—	—	—	—	—	—	2	—	6

dose of 200 or 250 mg. of kanamycin administered by aerosol inhalation can achieve therapeutic levels of the drug in bronchial secretions within 30 minutes to one hour after inhalation. On the other hand, low or no detectable levels were found in the bronchial secretions after the intramuscular administration of kanamycin regardless of the height of the serum levels. In the case of P. B., the serum concentration reached 112.0  $\mu\text{g./ml.}$ , but there was no detectable level in the bronchial secretions. In only 1 case were the bronchial and serum concentrations approximately equal.

Drug toxicity such as skin rashes, albuminuria, elevation of blood urea nitrogen, or impairment of hearing was not observed in this group of patients treated with kanamycin. Blood prothrombin time was determined in 6 patients after continuous therapy for 7 to 10 days. Only 1 of 6 patients had a transient reduction of blood prothrombin level; this was normal when retested after an interval of three days after discontinuance of the drug. Whether this transient reduction of blood prothrombin level was related to kanamycin administration is not certain. Subsequent studies have shown that this depression of blood prothrombin is not due to the administration of kanamycin. Bronchoscopic examinations did not show evidence of irritation of the mucosa after aerosol therapy. Aerosol treatment did not cause any inflammatory changes in the nose or oropharynx.

#### DISCUSSION

The present study revealed that kanamycin is often effective in producing clinical improvement in chronic bronchopulmonary infections occurring in patients with fibrocystic disease of the pancreas. Most strains of staphylococci isolated from these patients are susceptible to kanamycin. This drug appears to be a good therapeutic agent in those patients in whom staphylococci are the predominant pathogens in the bronchial secretions. Kanamycin may be administered concurrently with other antimicrobial agents to reduce the development of resistance of staphylococci against kanamycin.

Four of the 13 patients in this series had *Ps. aeruginosa* isolated from the bronchial secretions. Most of these strains were resistant to kanamycin. These patients

TABLE VI

*Kanamycin Concentrations in Serum and in Bronchial Secretions after Aerosol Inhalation of Kanamycin*

Patient	Weight, Kg.	Kanamycin, mg. by aerosol	Time interval, hr.	Serum, $\mu\text{g./ml.}$	Bronchial, $\mu\text{g./ml.}$
M. J.	14	200	1	0	12.5
C. O.	22	200	1½	0	47.0
C. O.	22	250	½	1.5	50+
S. F.	10	250	1	Trace	34.0

TABLE VII  
Kanamycin Concentrations in Serum and in Bronchial Secretions after the  
Intramuscular Administration of Kanamycin

Patient	Weight, Kg.	Kanamycin, mg./Kg. intramuscularly	Time interval, hr.	Serum, μg./ml.	Bronchial, μg./ml.
M. J. M.	15	20	2½	13	10
J. V.	12	50	2½	35.6	0
P. B.	13	40	1¼	112.0	0
L. A.	16	30	2½	70.0	7
S. F.	10	50	3	36.8	Trace
R. K.	20	25	2	60.4	8

improved clinically on kanamycin therapy and bronchoscopic drainage. Similar results have been observed in the same patients after penicillin and streptomycin treatment, although there was laboratory evidence of bacterial resistance to these two antibiotics. Bronchoscopic aspiration must account for some of the improvement. Kanamycin is not recommended in patients who harbor *Ps. aeruginosa* as the only pathogen.

There is poor diffusion of kanamycin from the serum into the bronchial secretions. Therefore, there is no constant relationship between the levels of kanamycin in the serum and in the bronchial secretions. Administration of kanamycin by both intramuscular injection and aerosol inhalation should provide therapeutic levels in both serum and bronchial secretions. In a few children kanamycin has been administered by aerosol inhalation for prolonged periods without adverse effects. Kanamycin has been used combined with pancreatic dornase and bronchodilators for aerosol inhalation.

#### SUMMARY

1. Thirteen patients with chronic pulmonary infections secondary to fibrocystic disease of the pancreas were treated with 17 courses of kanamycin therapy administered by the intramuscular route and 14 courses of kanamycin aerosol inhalation.

2. All the patients showed some evidence of clinical improvement, and none became worse during the course of treatment.

3. No toxic reactions have been observed after both intramuscular and aerosol therapy in these patients.

4. The pathogens isolated from these patients were predominantly coagulase-positive, hemolytic *Staph. aureus* and *Ps. aeruginosa*.

5. Considerably higher concentrations of kanamycin were detected in the bronchial secretion than in the serum after aerosol inhalation of the drug, and the reverse was found after intramuscular administration.

6. These findings suggest that kanamycin administered concurrently by the aerosol and intramuscular routes will be beneficial in many patients with chronic bronchopulmonary infections superimposed on fibrocystic disease of the pancreas.

#### BIBLIOGRAPHY

1. HUANG, N. N., AND HIGH, R. H.: Antibiotic-resistant staphylococci isolated from patients in a children's hospital. In: Antibiotics Annual 1954-1955, New York, Medical Encyclopedia, Inc., 1955, p. 1085.
2. GROVE, D. C., AND RANDALL, W. A.: Assay Methods of Antibiotics: A Laboratory Manual, New York, Medical Encyclopedia, Inc., 1955, p. 34.

# The Effect of Long-Term Antibiotic Therapy in Patients with Cystic Fibrosis of the Pancreas

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Cystic fibrosis is now being recognized with increasing frequency. The number of patients seen at the Children's Medical Center indicates that this is not a rare disease. In 1947, the number of living patients known to the clinic was approximately 40, whereas in 1957, more than 90 new patients were admitted. Although the true incidence is not known, it is estimated that approximately 1 of every 700 children born in this country may develop cystic fibrosis.

The pulmonary involvement is the greatest handicap and life threat for the patient with cystic fibrosis. The production of thick, mucous secretions and superimposed infection occur in the majority of patients. The extent, severity, and progress of the pulmonary infection determine the prognosis. The clinical picture may resemble staphylococcal pneumonia, chronic bronchitis, asthmatic bronchitis, lobar or lobular atelectasis, bronchopneumonia, or bronchiectasis. Reference need not be made here to the nutritional failure, which may be secondary to pancreatic enzyme deficiency or to chronic pulmonary infection.

The prognosis in untreated patients is extremely poor. Inasmuch as there is considerable variation in the severity of the pulmonary process, it is necessary to deal with large groups of patients or with comparable individual cases in determining the effectiveness of any therapeutic program. A method has been described recently for assessing the severity of the disease on clinical grounds, and data on 105 patients treated and observed more than five years have been reported.<sup>1</sup> Forty-one of these patients were more than 10 years of age at the time of that report,<sup>1</sup> whereas prior to the advent of broad-spectrum antibiotics in late 1948, the average age at death of those who succumbed at the Children's Medical Center was 12 months. During the past five years the average age at death of 40 patients with cystic fibrosis was approximately 6 years. Although new and simple diagnostic tests have been introduced,<sup>2</sup> and a better understanding of various manifestations of this disease has developed, these differences in survival rates are not primarily a reflection of such advances, but rather of the effectiveness of antibiotic and supportive therapy.

This report deals with 50 patients treated with broad-spectrum antibiotics for a minimal period of eight years. Prior to the advent of chlortetracycline, sulfonamides were often given prophylactically for prolonged periods, and combinations of penicillin and streptomycin were used during acute exacerbations of pulmonary infection. Chlortetracycline\* was first administered in the fall of 1948 in a dosage schedule aimed at achieving a favorable clinical response with the smallest effective dosage. In small infants, for example, as little as 50 mg. given once a day was

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found to have a striking beneficial effect on the respiratory involvement in cystic fibrosis. The average dosage was calculated to be between 10 and 25 mg./Kg. administered either once or twice a day. In this study, 35 patients who attended the clinic in 1948 were given chlortetracycline on a continuous basis, and the clinical response was usually satisfactory. When oxytetracycline was introduced in Janu-

TABLE I  
*Long-Term Antibiotic Therapy in 50 Patients with Cystic Fibrosis*

Pt.	Antibiotic*						Duration of therapy, yr.
	Chlor-tetracycline	Oxytetra-cycline	Chlor-amphenicol	Erythro-mycin	Tetra-cycline	Novo-biocin	
1	4	2	2	2			8
2	6		2	2			8
3	3	3½	2½	2½			9
4	6	3					9
5	7	2					9
6	2½	3½	3	3			9
7	6				2		8
8	5 + 1	3		1			9
9	2 + 2	1½	4½	2½		1	8
10	6 + 2	1		2			9
11	7				1		8
12	6 + 1		2	1			8
13	8						8
14	½	8		½			8½
15	4	2 + 1½		1½	2		9½
16	2 + 1½	2½	2	2			8
17	4 + 1		3	4			8
18	2 + ½	3	2	3			8½
19	2	3½ + 1	1½	2			8
20	5	2	1	1			8
21	4	3	1	1			8
22	1 + 1½	3½	3	2½			8
23	6 + 3			3			9
24	7½	½	½				8½
25	6½	1	2	2			8
26	2½	2½ + 1½	3	1½			8
27	½	4			1½ + 2		8
28	9½						9½
29	6	3					9
30	6			2	2		8
31	4		1½	1½	2½		8
32	2½	3	2½	2½			8
33	3 + 2	2	1½	3½			8½
34	5½	2½					8
35	4 + 2	1	2	2			8
36	3½ + 3	2		3			8½
37	8						8
38	8		1	1			9
39	1½	7					8½
40	2½	5½					8
41	4 + 1½	2½		1½			8
42	6	2					8
43	4½ + 2½		1½	4			8½
44	2½ + 1½	2½	3	1½			8
45	7 + 1			1			8
46	3½ + 1	2½	2	1			8
47	1½	3½	2	2			8
48	4½ + ½	2	2	1	½		8½
49	4	1	1	3		2	8
50	5	1	2				8

\* In those instances where combination therapy was used the antibiotics are italicized, e.g., patient 10 received chlortetracycline for a total of six years, during which time erythromycin also was used in combination for two years.

TABLE II

*Comparison of Initial and Present Ratings of 50 Patients Receiving Continuous Antibiotic Therapy for a Period of 8+ Years*

Living	Dead	No. patients	Rating*				
			Excellent	Good	Mild	Moderate	Severe
Initial		43	1	1	18	22	1
Present		43	3	16	19	4	1
	Initial	7			2	5	

\* See reference 1.

ary, 1950, a similar dosage schedule was employed in 15 patients with equal effectiveness.

Table I summarizes the antibiotic therapy employed in the care of these 50 patients. The use of antibiotics in this manner had a very beneficial effect, not only in improving the pulmonary status, but also in permitting initial weight gain. No serious toxic effects were noted, and these drugs were used continuously, since it was found that without such therapy these children had recurrences of the pulmonary symptoms and exacerbations of the pulmonary infections.

It is interesting to speculate whether such beneficial effects are due primarily to the antibacterial activity of the antibiotic or occur for some other reason. In many patients the pulmonary process advances despite continuous antibiotic therapy, even at increased dosage, with or without other antibacterial agents (sulfonamides, erythromycin, chloramphenicol, or novobiocin), or despite the use of aerosol therapy with penicillin, streptomycin, neomycin, or polymyxin. Other therapeutic measures included the use of iodides, bronchodilators, mist therapy, postural drainage, and physiotherapy to improve pulmonary ventilation, as well as dietary measures based on a high-protein, low-fat diet, including pancreatic replacement therapy and vitamin supplements. Experience has indicated that in general, clinical appraisal, including chest roentgenograms, is a better guide to antibiotic therapy than is bacteriological assessment of the nasopharyngeal flora. The severity of the disease may be estimated and rated on clinical grounds, and in this way one may follow the course of the illness in any one patient, as well as make comparisons between patients. A patient with a score of 85 or more, as judged by the previously described criteria,<sup>1</sup> is considered in excellent condition, whereas a patient with a score of 40 or less is considered to be severely ill.

The status of the patients in the present group as determined by this rating sys-

TABLE III

*Side Reactions Observed during Continuous Prophylactic Antibiotic Therapy in Children with Cystic Fibrosis*

Reaction	No. patients
Diarrhea	2
Photosensitivity	5
Dark staining of teeth	40±
Angular stomatitis	8
Skin rash	4*

\* After novobiocin in three instances.

FIG. 1. Shown is an example of staining of the teeth.



tem is summarized in table II, wherein a comparison is made of their initial and present ratings. Among this group of 50 patients, 7 have succumbed after eight or more years of antibiotic treatment. The condition of these 7 patients when just seen was considered mild in 2 and moderate in 5 cases. The current condition of the remaining 43 patients shows an over-all improvement. In a comparison of the initial and present clinical rating, 28 patients are better, 13 are unchanged, and 2 are worse. In general, the tolerance of the antibiotics in the small dosages used in this study was good. Table III lists the side reactions encountered. Staining of the teeth is illustrated in figure 1.

The age at diagnosis and the present ages of the patients in this study are shown in table IV. Although the majority of patients (62 per cent) were diagnosed prior to 2 years of age, 64 per cent are now more than 10 years of age. These survival figures become even more impressive when compared with the studies reported by Bodian,<sup>3</sup> in which 53 of 68 fatal cases among 116 patients were under 2 years of age, and in which the majority of patients were dead one year after the diagnosis was established.

Bacteriological studies of the nasopharyngeal flora of patients with cystic fibrosis, the effects of antibiotic therapy on such flora, and the species of microorganisms isolated prior to and after antibiotic therapy have been reported from these laboratories.<sup>4-6</sup> A summary of these findings follows: More than 90 per cent of untreated patients with cystic fibrosis harbor *Micrococcus pyogenes* var. *aureus*.

TABLE IV  
Age at Diagnosis and Present Age of 50 Patients with Cystic Fibrosis  
Receiving Continuous Antibiotic Therapy

Age at diagnosis	No. patients		Present age of patients (yr.), Sept. 1, 1958		
	Living	Dead	8-10	10-15	More than 15
Birth*	4	1	4 (1)		
1 wk.-6 mo.	10	2	8 (2)	3	
6 mo.-2 yr.	12	2	3 (1)	9 (1)	
2-4 yr.	8	2	1 (1)	4 (1)	2
More than 4 yr.	9			4	5
Total	43	7	16 (5)	20 (2)	7

Figures in parentheses indicate patients who died.  
\* Meconium ileus.

TABLE V  
*Nasopharyngeal Flora in 50 Patients with Cystic Fibrosis Receiving  
Continuous Antibiotic Therapy*

Therapy	No. of cultures	<i>M. pyogenes</i> var. <i>aureus</i> , %	<i>Bacillus proteus</i> var. <i>vulgaris</i> , %	<i>Ps. aeruginosa</i> , %
None*	27	92.5		
After penicillin and streptomycin and sulfonamides	27	66.5	29.5	13
After short-term broad-spectrum antibiotics, 2 wk.-2 yr.	50	88	18	10
After long-term broad-spectrum antibiotics, 8+ yr.	40	72.5	30	5

\* Prior to antibiotics.

In 1948, prior to the use of chlortetracycline and after the use of penicillin and streptomycin, the in vitro sensitivity of *M. pyogenes* var. *aureus* isolated from 42 patients with cystic fibrosis was as follows: 88 per cent were sensitive to less than 4  $\mu$ g./ml. of chlortetracycline, 66 per cent were sensitive to less than 4  $\mu$ g./ml. of streptomycin, and 26 per cent were sensitive to less than 4 Oxford units/ml. of penicillin. The flora of the nose and throat was essentially unchanged in many patients after many months of therapy with chlortetracycline. Clinical improvement was observed frequently despite the repeated isolation of *M. pyogenes* var. *aureus* that were resistant to chlortetracycline. Similar observations were made with oxytetracycline, which was found to be equal in clinical effectiveness.

A summary of recent cultures taken after eight to nine and one-half years of antibiotic therapy is presented in table V, which also includes some of these earlier observations. Antibiotic therapy perhaps has reduced the frequency of, but has by no means eliminated, *M. pyogenes* var. *aureus* from the nasopharyngeal flora of these patients (table V), despite the remarkable improvement in clinical condition and life expectancy that has resulted from continuous antibiotic therapy. The use of broad-spectrum antibiotic therapy, aside from its questionable effect on the presence of *M. pyogenes* var. *aureus*, has been associated with an increased frequency of gram-negative species in the nasopharyngeal flora (table V). This

TABLE VI  
*Frequency of Antibiotic-Resistant Microorganisms Isolated from the Nasopharynx of Patients*

Antibiotic	Concentration	<i>M. pyogenes</i> var. <i>aureus</i> , resistant	
		1948*	1957-1958†
Penicillin	5 Oxford units	74‡	68
Streptomycin	10 gamma	44§	40
Chlortetracycline	10 gamma	12	89
Erythromycin	15 gamma		18
Chloramphenicol	10 gamma		4
Neomycin	10 gamma		0

\* Forty-two strains from 42 patients. Eighty-eight per cent of the strains isolated were sensitive to less than 4 gamma.

† Twenty-eight strains from 50 patients.

‡ Twenty-six strains from 42 patients.

§ Twenty-five strains from 42 patients.

TABLE VII

*Bacteriophage Type of M. pyogenes, var. aureus Isolated from Nasopharynx of 29 Patients with Cystic Fibrosis*

Bacteriophage type	No. of strains	No. of patients*
I	0	
(80-81)	12 + 2 postmortem	7
II	0	
III	20	15
IV	0	
Mixed	3	3
Nontypable	15	11 + 1 postmortem
Total	50 + 2 postmortem	36 + 1 postmortem

\* Sixteen patients typed once, 13 patients typed repeatedly (see table VIII).

change has also been reflected in a similar change in the flora associated with the terminal pulmonary infection that invariably occurs in the fatal case. Whereas prior to the use of antibiotic therapy, the terminal infection was invariably associated with *M. pyogenes* var. *aureus* (often recovered in pure culture at autopsy), these events now are usually associated with gram-negative microorganisms,<sup>7</sup> particularly an encapsulated hemolytic variety of *Pseudomonas aeruginosa*.<sup>8</sup>

The 28 strains of *M. pyogenes* var. *aureus* isolated during the past year from the nasopharyngeal flora of these patients were tested for sensitivity against six antibiotics by a disc method;<sup>9</sup> the results are summarized in table VI. Eighty-nine per cent of the 28 strains of *M. pyogenes* var. *aureus* that were examined during the year 1957-1958 were resistant to 10 gamma of chlortetracycline, as compared with 12 per cent of the strains examined in 1948 that exhibited a comparable degree of resistance. It is of interest to note (table VI) that the frequency of peni-

TABLE VIII

*Bacteriophage Type of M. pyogenes var. aureus Recovered from Repeated Nasopharyngeal Cultures in the Same Patients*

Patient	Initial	Repeat cultures				Total elapsed time, mo.
		1	2	3	4	
33	III <sup>1</sup>	III <sup>1</sup>	III <sup>1</sup>	III <sup>1</sup>		7
13	Nontypable	Nontypable	Nontypable	Nontypable		9
48	80, 81	80, 81	80, 81	80, 81*		3
49	III <sup>2</sup>	III <sup>2</sup>	III <sup>2</sup>	III <sup>2</sup>		2
26	80, 81	80, 81				3
42	80, 81	80, 81				8
2	III <sup>3</sup>	I and II <sup>4</sup>	80, 81			10
39	III <sup>2</sup>	Nontypable				4
22	III <sup>5</sup>	Nontypable				8
16	III <sup>6</sup>	III <sup>6</sup>	III <sup>6</sup>	Nontypable		5
30	III <sup>7</sup>	III <sup>7</sup> and IV				10
38	80, 81	80, 81	Nontypable			11
44	III <sup>8</sup>	80, 81	80, 81*			9

\* Also recovered from lungs at autopsy.

<sup>1</sup> Phage type: 47, 54.

<sup>2</sup> Phage type: 53, 77, 47, VA4, 6.

<sup>3</sup> Phage type: 6, 7, 47, 53.

<sup>4</sup> Phage type: 29, 52A, 79, 6, 7, 42E, 54, 73, 47/c.

<sup>5</sup> Phage type: 47, 54, 77.

<sup>6</sup> Phage type: 6.

<sup>7</sup> Phage type: 6, 7.

<sup>8</sup> Phage type: 6, 42E, 47, 53, 54, 77, VA4.

cillin-resistant strains isolated from patients with cystic fibrosis during 1957–1958 did not differ materially from the frequency with which such strains were encountered in the 1948 studies<sup>1</sup> (table VI). Similarly, the frequency of antibiotic-resistant strains of *Ps. aeruginosa* encountered in 1957–1958 was no greater than that observed in the 1948 studies.

During the past year, 25 strains of *M. pyogenes* var. *aureus* isolated from the nasopharyngeal flora of patients with cystic fibrosis have been studied with kanamycin in vitro and 24 were sensitive to the 10  $\mu$ g. disc. Of five strains of *Ps. aeruginosa* so studied, only one was sensitive to the 10  $\mu$ g. disc.

The bacteriophage type of the strains of *M. pyogenes* var. *aureus* isolated from those patients studied subsequent to June, 1957, has been determined. Since that time, 52 strains isolated from 29 patients have been so studied; the results are summarized in table VII. It is apparent that no single bacteriophage type is peculiar to these patients. However, 7 patients harbored the so-called epidemic strain (80, 81), and in none of these patients was there evidence of dissemination of the infection.

The bacteriophage types of the strains of *M. pyogenes* var. *aureus* isolated from repeat cultures on 13 patients over a period of 11 months at three to four week intervals are summarized in table VIII. Although it appears that in most instances the individual patient tends to harbor the same bacteriophage type for a considerable period of time, there may be some changes in flora, as illustrated by the last 7 patients (table VII).

#### SUMMARY

Cystic fibrosis of the pancreas, primarily a disease of childhood, is being recognized with increasing frequency. More than 90 newly diagnosed cases were seen in our clinic in 1957.

The results of a long-term study of 50 patients treated with broad-spectrum antibiotics for a minimal period of eight years are presented. Among this group, 7 succumbed after at least eight years of antibiotic therapy. Minimal doses given continuously were found effective in arresting the pulmonary infection in the majority of patients. Details of dosage schedule, clinical assessment of patients, and untoward reactions are presented. Of the 43 survivors, 28 patients are now better than on the initial visit, 13 are unchanged, and 2 are worse. Sixty-four per cent of this group of patients are now more than 10 years old. The tolerance to the antibiotics was good with few side reactions. Staining of the teeth occurred in approximately 80 per cent of the patients.

Bacteriological studies include observations of the nasopharyngeal flora prior to and after antibiotic therapy. The initial cultures revealed *M. pyogenes* var. *aureus* in more than 90 per cent of the patients. After prolonged therapy, the incidence of *M. pyogenes* var. *aureus* was reduced to approximately 70 per cent with the concomitant appearance of gram-negative species. Sensitivity studies of the *M. pyogenes* var. *aureus* obtained prior to therapy with chlortetracycline indicated that approximately 12 per cent of the strains were resistant to 4 gamma of chlortetracycline as compared with an incidence of 90 per cent of resistant strains to 10 gamma chlortetracycline isolated in 1957–1958. The bacteriophage type of 52 strains of *M. pyogenes* var. *aureus* isolated from 29 patients studied subsequent to June, 1957, is presented. No single phage type is peculiar to these patients. In 7 patients, strain 80, 81 was isolated, and in 6 of these, on more than one occasion.

## BIBLIOGRAPHY

1. SHWACHMAN, H., AND KULCZYCKI, L. L.: Long term study of 105 patients with cystic fibrosis. *J. Dis. Child.* 96:6, 1958.
2. SHWACHMAN, H., AND GAHM, N.: Studies in cystic fibrosis of the pancreas: simple test for detection of excess chloride on skin, *New England J. Med.* 255:999, 1956.
3. BODIAN, M.: *Fibrocystic Disease of the Pancreas*, New York, Grune & Stratton, Inc., 1953.
4. SHWACHMAN, H.; CROCKER, A. C.; FOLEY, G. E., AND PATTERSON, P. R.: Aureomycin therapy in pulmonary involvement of pancreatic fibrosis (mucoviscidosis), *New England J. Med.* 241:185, 1949.
5. SHWACHMAN, H.; SILVERMAN, B. K., AND PATTERSON, P. R.: Antibiotics in treatment of pancreatic fibrosis with emphasis on terramycin, *J.A.M.A.* 149:1101, 1952.
6. STOPPELMAN, M. R. H., AND SHWACHMAN, H.: Effect of antibiotic therapy on mucoviscidosis, *New England J. Med.* 251:759, 1954.
7. FOLEY, G. E.; WINTER, W. D., JR., AND SHWACHMAN, H.: To be published.
8. FOLEY, G. E., AND SHWACHMAN, H.: To be published.
9. SHERR, G. H.: A new type of impregnated paper disc for determining microbial sensitivity to antibiotics and other chemotherapeutic agents, *Antib. & Chemo.* 4:1007, 1954.

# The Ototoxicity of Kanamycin in Man

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The introduction of a new antibiotic, closely related in chemical structure to streptomycin and neomycin, raises at once the question of its possible toxic effect on the sensory endings of the inner ear. Kanamycin, discovered in 1957 by Umezawa and associates,<sup>1</sup> has been claimed, on the basis of preliminary animal experiments,<sup>2</sup> to be less toxic for the vestibular system than streptomycin, and less toxic for the auditory system than dihydrostreptomycin and neomycin. The earliest clinical investigations<sup>3-6</sup> have appeared to support this conclusion, although the authors warn of the possibility of damage to the hearing. The results of long-term use to be reported here indicate that kanamycin is an ototoxic drug that should be administered with great caution.

Through the courtesy of McClement and his colleagues of the Bellevue Hospital Chest Service, we have had the opportunity of making audiometric studies in patients receiving prolonged kanamycin treatment for chronic pulmonary tuberculosis. Other aspects of this evaluation of kanamycin have recently been presented by McClement and associates.<sup>7</sup>

Ideally, such a study should be carried out in patients with normal auditory and vestibular function, and in such a way as to permit comparison with another group of similar patients receiving an antibiotic of known ototoxicity, i.e., streptomycin or dihydrostreptomycin. For valid clinical reasons, such conditions can seldom be justified in testing a new antitubercular agent. The 14 patients in the first group chosen to receive kanamycin had previously received treatment with most of the usual antitubercular drugs. Their organisms were already highly resistant to these agents, so that no therapeutic effect could be expected from further chemotherapy unless with a new drug such as kanamycin.

The group consisted of 8 men aged 30 to 60 years and 6 women aged 30 to 36 years.<sup>1</sup> (Of the 17 patients in the Bellevue study,<sup>7</sup> 2 were too ill for audiometric measurements to be made. A third, whose hearing became impaired during treatment, was found to have been receiving viomycin as well as kanamycin, and is therefore excluded from this report.) All had received prolonged treatment in other hospitals with streptomycin and/or dihydrostreptomycin, as well as with isoniazid and *p*-aminosalicylic acid. Three had received viomycin and three, cycloserine, but none had received neomycin. Each patient was given a complete otological examination before treatment was started. No indication of middle ear disease was found. Seven patients still had normal audiograms, i.e., no deviation greater than 15 decibels from the "average hearing" (0 decibels) abscissa, at the time kanamycin treatment was started. Two had moderate losses (30 to 35 decibels) at 8000 cycles/sec. only. Two others had similar losses at 4000 and 8000 cycles/sec. and 3 had losses at 2000, 4000, and 8000 cycles/sec. Only 1 patient (P. A.) complained of tinnitus before receiving kanamycin (table I).

Of the second group of 8 patients, only 1 (R. F.) had received any previous chemotherapy for tuberculosis. Four had normal audiograms before treatment. One had a slight bilateral loss at 4000 and 8000 cycles/sec., another a more extensive

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TABLE I  
*Hearing Levels in First Group of Patients Before Treatment with Kanamycin*

Patient	Sex Age, yr.	Dose		Previous treatment* with viomycin	Pre-kanamycin hearing levels, decibels, at cycles/sec.		
		Gm./day	mg./Kg.		2000	4000	8000
P. A.	F 30	0.5	13	—	R 35 L 40	30 40	35 40
C. S.	F 30	0.5	11	Yes	R 15 L 5	15 5	10 5
B. S.	F 35	0.5	10	—	R 10 L 5	10 15	15 5
D. McC.	M 39	1.0	22	Yes	R 15 L 20	75 55	75 70
W. J.	M 43	1.0	14	—	R 40 L 30	30 30	45 30
M. C.	M 52	1.0	18	—	R 5 L 10	55 35	70 55
H. F.	M 31	1.0	17	Yes	R 10 L 5	10 5	20 15
J. P.	M 48	1.0	13	—	R 15 L 10	15 25	30 35
F. R.	M 30	1.0	14	—	R 10 L 0	15 10	15 30
E. J.	M 60	1.0	14	—	R 15 L 10	15 15	15 15
J. H.	M 42	1.0	18	—	R 15 L 5	15 10	30 35
E. M.	F 31	1.5	27	—	R 10 L 5	10 5	15 10
R. McL.	F 36	1.5	26	—	R 10 L 5	10 15	15 10
N. W.	F 34	1.5	39	—	R 10 L 5	5 10	15 15

\* All these patients had been treated previously with streptomycin/dihydrostreptomycin.

perceptive loss from 1000 to 8000 cycles/sec. The last patient showed a combined conductive and perceptive loss of 15 to 55 decibels, involving all frequencies (table II).

#### DRUGS AND REGIMENS

The kanamycin sulfate used in the study was supplied by Bristol Laboratories. It was stated to consist of kanamycin A, with only 1 to 3 per cent of kanamycin B. Only 1 patient (M. C.) received treatment with an early, less purified lot stated to contain 10 to 20 per cent of kanamycin B.

The antibiotic was given by intramuscular injection of an aqueous solution. Of the first group of patients, 3 received 0.5 Gm./day (10 to 13 mg./Kg.), 8 received 1.0 Gm./day (0.5 Gm. twice daily; 13 to 22 mg./Kg.), and the remaining three patients 1.5 Gm./day (0.75 Gm. twice daily; 26 to 39 mg./Kg.). Treatment was continued for at least 120 days, unless the appearance of hearing loss made it advisable to stop the drug.

All of the second group of patients received 1.0 Gm./day (0.5 Gm. twice daily; 15 to 25 mg./Kg.). Four are continuing treatment after 39 to 101 days. The others were stopped at 40 to 94 days as soon as any loss of hearing became evident.

#### HEARING LOSS

Losses of 15 decibels or more from the pretreatment audiogram were regarded as significant. Such losses occurred in all but 2 of the first group of patients, as shown in table III. The earliest complaint of difficulty in hearing was made after a

TABLE II  
*Hearing Levels in Second Group of Patients Before Treatment with Kanamycin*

Patient	Sex Age, yr.	Dose		Previous treat.* with streptomycin/ dihydrostrep- tomycin	Pre-kanamycin hearing levels, decibels, at cycles/sec.		
		Gm./day	mg./Kg.		2000	4000	8000
M. G.	M	1.0	15	No	R 0	5	5
	41				L 5	5	0
C. J.	M	1.0	25	No	R 5	5	5
	24				L 15	15	15
E. S.	M	1.0	17	No	R 15	20	35
	43				L 10	15	25
A. C.	F	1.0	20	Yes	R 10	10	0
	36				L 5	5	5
J. L.	M	1.0	16	No	R 10	40	65
	49				L 15	20	30
I. C.	M	1.0	19	No	R —5	10	10
	51				L 5	15	15
C. T.	M	1.0	15	No	R 35	45	55
	51				L 35	40	55
R. F.	F	1.0	21	No	R 5	10	10
	33				L 5	10	5

\* None of these patients had been treated with viomycin.

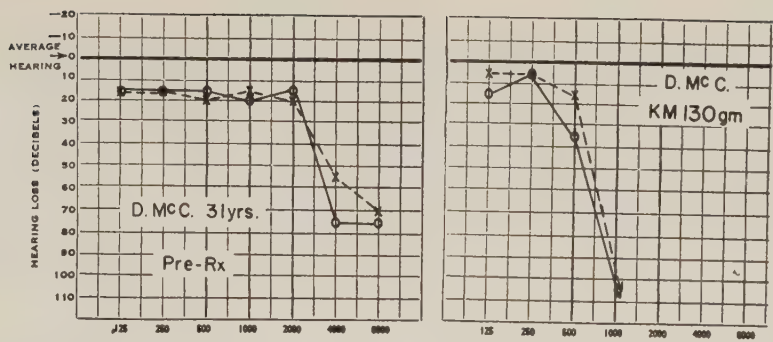
total dose of 32 Gm. by patient P. A., who already had a hearing loss and tinnitus and was receiving only 0.5 Gm./day. An audiogram taken after 43 Gm. total dose showed a further loss at 2000 to 8000 cycles/sec., which increased in severity during the following month of chemotherapy but did not involve frequencies less than 2000 cycles/sec. The 2 other patients did not develop any hearing loss.

TABLE III  
*Summary of Hearing Losses in First Group of Patients*

Patient	Total Gm. kanamycin at onset		Total dose, Gm.	Post-kanamycin hearing levels, decibels, at cycles/sec.					
	Hearing loss	Tinnitus		500	1000	2000	4000	8000	
P. A.	32	Pre-treatment	60	R 10	5	50	60	70	
				L 10	10	55	65	*	
C. S.	—	—	61	R 5	5	10	5	10	
				L 10	5	5	0	5	
B. S.	—	—	59	R —5	0	—5	—5	5	
				L 5	0	0	5	5	
D. McC.	56	40	130	R 35	*	*	*	*	
				L 15	*	*	*	*	
W. J.	101	95	112	R 5	20	75	90	*	
				L 5	20	45	45	45	
M. C.	95	95	122	R 20	15	50	*	*	
				L 25	20	50	80	—	
H. F.	121	—	163	R 0	0	—5	0	10	
				L 0	0	0	0	45	
J. P.	103	—	163	R 25	25	70	75	*	
				L 15	15	30	70	*	
F. R.	82	70	142	R 5	5	25	45	45	
				L 15	5	10	65	80	
E. J.	101	—	120	R 0	0	15	15	45	
				L 0	5	10	25	60	
J. H.	50	—	128	R 5	5	5	15	45	
				L 5	5	5	25	45	
E. M.	54	72	143	R 10	5	35	*	*	
				L 15	40	*	*	*	
R. McI.	123	118	154	R 0	5	40	40	45	
				L 5	—5	—5	10	50	
N. W.	134	134	149	R 5	5	5	45	80	
				L 15	0	0	55	75	

\* Not heard.

FIG. 1. Hearing loss in D. McC., age 31 years.



Among the patients receiving 1.0 Gm./day, all showed some degree of loss, ranging from a slight deficit of 10 to 15 decibels at 4000 and 8000 cycles/sec. in the case of patient J. H. to a profound deafness in the case of patient D. McC. (fig. 1). Two others, W. J. and M. C., complained of difficulty in hearing and 1 of them refused further treatment on that account. Total dose at the time the loss was first recognized, either audiometrically or subjectively, varied from 56 to 121 Gm.

All 3 of the patients receiving 1.5 Gm./day showed losses, although they were not so severe as some that occurred at 1.0 Gm./day. The earliest complaint of hearing difficulty was made after 54 Gm. by patient E. M., who reported that she could no longer hear her watch tick. In her case, and in that of R. McI., the loss was more extensive in one ear than in the other (fig. 2), while in patient N. W. it was symmetrical and confined to 4000 and 8000 cycles/sec., without involving the speech frequencies.

Four of the second group of patients are still receiving kanamycin therapy. The other 4 have had to be taken off the drug because of hearing loss. In 3 of these the loss began early, after only 40, 51, and 53 Gm. respectively, and in the fourth after 94 Gm. In each case kanamycin was stopped immediately in the hope of preventing further deterioration of the hearing (table IV).

TINNITUS

In the first group of patients, 8 out of the 12 showing hearing loss also reported tinnitus. One patient (P. A.), whose tinnitus was already present before the kanamycin treatment was begun, reported that it became worse during treatment. Another patient (E. M.) noticed tinnitus more than two weeks after she had first reported that her hearing was impaired. In all other cases the tinnitus accompanied or preceded the onset of hearing loss.

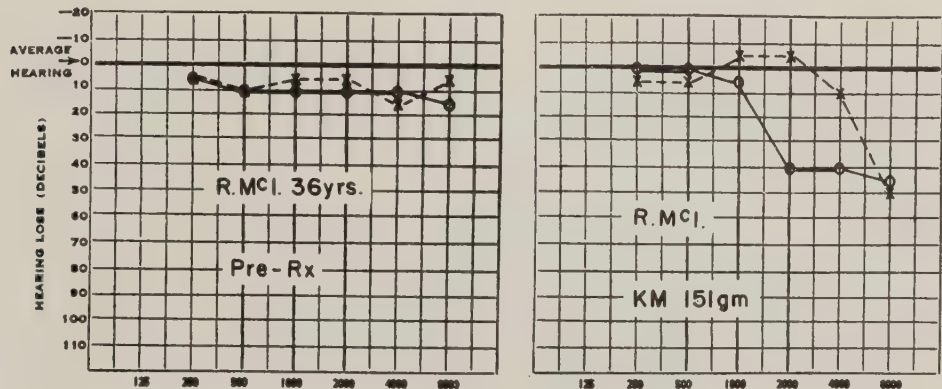


FIG. 2. Hearing loss in R. McI., age 36 years.

TABLE IV  
Summary of Hearing Losses in Second Group of Patients

Patient	Gm. of kanamycin at onset		Total dose, Gm.		Post-kanamycin hearing levels, decibels, at cycles/sec.				
	Hearing loss	Tinnitus			500	1000	2000	4000	8000
M. G.	—	—	113	R	10	10	5	5	5
				L	10	5	5	5	15
C. J.	94	—	94	R	5	5	5	0	45
				L	15	15	20	20	45
E. S.	—	—	63	R	0	5	5	15	25
				L	—5	—5	0	10	10
A. C.	51	45	51	R	5	0	5	5	45
				L	5	5	5	10	50
J. L.	—	—	49	R	15	15	10	45	45
				L	5	0	5	25	40
I. C.	53	50	53	R	20	20	10	30	65
				L	20	10	5	65	65
C. T.	40	40	40	R	*	*	*	*	*
				L	*	*	*	*	*
R. F.	—	—	63	R	0	0	—5	5	5
				L	5	0	0	5	5

\* Not heard.

Of the 4 patients in the second group showing hearing loss, 2 reported tinnitus a few days before their hearing loss was detected, 1 reported it on the same day, and 1 exhibited a loss at 8000 cycles/sec. without tinnitus.

### COURSE OF THE HEARING LOSS

In the first group, in which treatment was not stopped as soon as the hearing was

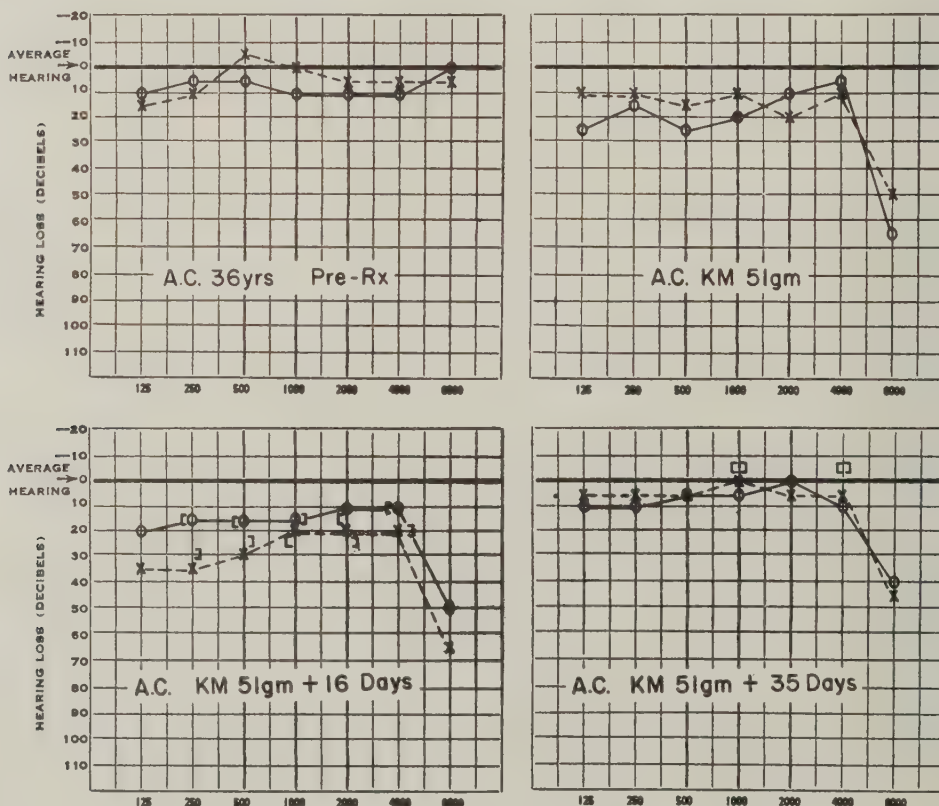


Fig. 3. Hearing loss after partial recovery in A. C., age 36 years.

affected, the impairment usually became worse as treatment was continued. It remained stationary during a two to four month follow-up period after treatment was stopped. In 1 case in the second group (A. C.) a flat perceptible loss, which appeared at all frequencies below 8000 cycles/sec. and was confirmed by two subsequent audiograms, actually reversed itself five weeks after kanamycin was stopped, so that only the loss at 8000 cycles/sec. remained (fig. 3). In another case (C. T.) of the second group, which is discussed below, the loss progressed after kanamycin was stopped and the patient become completely deaf (fig. 4).

RELATION TO NEPHROTOXICITY

Granular casts appeared in the urine of all patients during kanamycin treatment, but disappeared when the antibiotic was stopped. In only 1 case (C. T.) did signs of a more severe nephrotoxic effect appear. This patient, whose initial audiogram revealed a mild, bilateral, mixed type of hearing loss also showed 1+ to 3+ albumen in the urine prior to treatment. The blood urea nitrogen was 18 mg./100 ml. He was diagnosed as having glomerulonephritis as well as diabetes and pulmonary tuberculosis. Following 40 Gm. of kanamycin (0.5 Gm. twice daily; 15 mg./Kg. daily) his hearing quite suddenly and rapidly deteriorated. At the same time the blood urea nitrogen was found to have risen to 82 mg./100 ml. Plasma levels of kanamycin must also have been very high, but unfortunately were not measured. Although the drug was immediately discontinued, the hearing impairment became progressively worse over the following three weeks, so that eventually both ears were totally deaf.

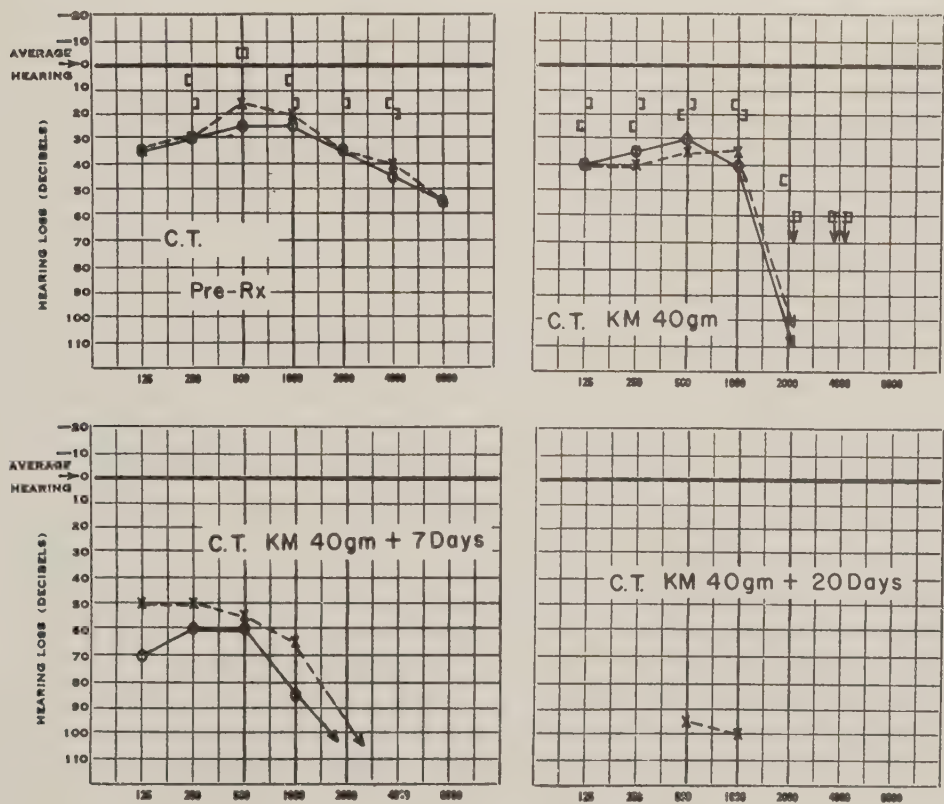


FIG. 4. Hearing loss in C. T., age 51 years.

Although several of the patients of the first group gave abnormal responses to caloric stimulation of the labyrinths, presumably as a result of previous treatment with streptomycin, C. T. was the only patient in either group who exhibited a disturbance of equilibrium as a result of kanamycin treatment. Several days following the onset of the hearing loss his gait was seen to be ataxic. This condition has now become so severe that he is unable to walk without assistance. Caloric and rotation tests, however, do not reveal any loss of function of the semicircular canals, and neurological findings are normal. This unusual case is being studied further.

## DISCUSSION

From this small series of patients it is clear that kanamycin possesses a substantial cochlear toxicity. Although no observations were made with dihydrostreptomycin in this study, the findings with kanamycin may be compared with those of Cohen et al<sup>8</sup> and Mahady et al,<sup>9</sup> who studied the ototoxicity of streptomycin and dihydrostreptomycin in tuberculous patients, using doses of 1.0 Gm. daily for 120 days or longer. In the series of Cohen et al 24 patients out of 189 (12.7 per cent) showed some degree of auditory toxicity while receiving a total dose of 120 Gm. of dihydrostreptomycin; in that of Mahady et al the figure was 12 patients out of 84 (14.3 per cent). With streptomycin the corresponding figures were 13 out of 195 (6.7 per cent) and 2 out of 82 (2.5 per cent). In the present study, 9 out of 14 patients (64 per cent) in the first group developed some degree of hearing loss while receiving less than 120 Gm. of kanamycin, and 4 out of 8 patients (50 per cent) in the second group. The incidence of hearing loss due to kanamycin is therefore considerably higher than that caused by dihydrostreptomycin in the other studies. The severity of the hearing loss is more difficult to compare because of differences in the initial audiograms, in previous therapy, and in the criteria for stopping treatment. It should be noted that in neither of the streptomycin-dihydrostreptomycin studies did losses occur as severe as of those patients D. McC. and C. T. in the kanamycin series. On the other hand, only 1 of the kanamycin patients (C. T.) has shown a progressive loss of hearing after treatment was stopped, whereas 36 of the 189 dihydrostreptomycin-treated patients in the study by Cohen et al developed tinnitus or hearing loss only after the drug was discontinued.

Neomycin, because of its severe cochlear toxicity, has been administered to very few patients by the parenteral route since the early studies of Carr et al<sup>10</sup> and of Waisbren and Spink.<sup>11</sup> In Carr's series, 4 out of 6 patients receiving neomycin (0.25 to 1.0 Gm. every 12 hours) were partially deaf after four to six weeks, with a progressive loss of hearing that became almost complete, even though treatment was stopped immediately. Waisbren and Spink reported deafness in 5 of their 63 cases treated with 1.5 to 2.0 Gm. daily for only 4 to 16 days, with the earliest loss appearing after seven days. Since the earliest loss with kanamycin occurred after 40 days, its ototoxicity must be less than that of neomycin, a conclusion that is supported by comparison of the two antibiotics in experimental animals.<sup>12</sup>

The place of kanamycin with respect to viomycin is more difficult to assess. In early clinical reports viomycin was said to show both vestibular and cochlear toxicity. Incidence of impairment of response to high frequencies was estimated at 20 to 30 per cent. Three cases of sudden, total deafness occurred among some 200 cases treated in various hospitals. The degree of toxicity appeared to vary considerably from one lot of viomycin to another. It is not clear that kanamycin shows any

important advantage over the best lots of viomycin so far as cochlear toxicity is concerned.<sup>13</sup>

Whether patients with a pre-existing perceptive deafness are more sensitive to kanamycin toxicity cannot be stated with certainty, but it appears likely from the present data. With one exception (E. M.), all of the patients in the first group showing the more severe losses after kanamycin had some degree of loss before kanamycin was started. In the second group, 2 patients who developed hearing loss had abnormal audiograms before treatment and 2 did not. In any case, it now appears advisable to exercise special caution in administering kanamycin to patients with hearing loss, especially those whose loss may be due to previous antibiotic treatment.

#### SUMMARY AND CONCLUSIONS

1. Kanamycin produced impairment of hearing in 16 of 22 patients given 0.5 to 1.5 Gm. daily for 120 to 163 days. In one case showing severe renal effects, total loss of hearing occurred after only 40 Gm. of the antibiotic had been given. Severe ataxia also occurred in this patient but in none of the others.

2. Kanamycin has a greater cochlear toxicity than dihydrostreptomycin but less than neomycin.

3. If kanamycin is to be given for more than 10 days, a pretreatment audiogram should be obtained, and audiograms taken at weekly intervals during treatment. Special caution should be observed in using the antibiotic to treat patients who already have a perceptive hearing loss or impaired renal function.

#### BIBLIOGRAPHY

1. UMEZAWA, H.; UEDA, M.; MAEDA, K.; YAGISHITA, K.; KONDO, S.; OKAMI, Y.; UTAHARA, R.; OSATO, Y.; NITTA, K., AND TAKEUCHI, T.: Production and isolation of a new antibiotic, kanamycin, *J. Antib., Ser. A.* 10:181-188, 1957.
2. TISCH, D. E.; HUFTALEN, J. B., AND DICKISON, H. L.: Pharmacological studies with kanamycin, *Ann. New York Acad. Sc.* 76:44-65, 1958.
3. DONOMAE, I.: Clinical studies of kanamycin treatment of pulmonary tuberculosis, *Ann. New York Acad. Sc.* 76:166-187, 1958.
4. ICHIKAWA, T.: Kanamycin treatment of urinary infections, *Ann. New York Acad. Sc.* 76:242-264, 1958.
5. BUNN, P. A.; BAITCH, A., AND KRAJNYAK, O.: Clinical experiences with kanamycin, *Ann. New York Acad. Sc.* 76:109-121, 1958.
6. DAVIES, F. G.: Clinical evaluation of the new antibiotic: kanamycin, *Ann. New York Acad. Sc.* 76:129-135, 1958.
7. BOWEN, J. F.; JONES, J. M.; NASH, E. S.; RILEY, E. A.; SIMPSON, D. G., AND McCLEMENT, J. H.: Clinical experience with kanamycin in chronic pulmonary tuberculosis, *Ann. New York Acad. Sc.* 76:163-165, 1958.
8. COHEN, S. S.; JOHNSON, L.; LICHTENSTEIN, M. R., AND LYNCH, W.: A comparative study of streptomycin and dihydrostreptomycin in pulmonary tuberculosis, *Am. Rev. Tuberc.* 68:229-237, 1953.
9. MAHADY, S. C. F.; ARMSTRONG, F. L.; BECK, F.; HORTON, R., AND LINCOLN, N. S.: A comparative study of streptomycin and dihydrostreptomycin in pulmonary tuberculosis, *Am. Rev. Tuberc.* 68:238-248, 1953.
10. CARR, D. T.; PFUETZE, K. H.; BROWN, H. A.; DOUGLAS, B. E., AND KARLSON, A. G.: Neomycin in clinical tuberculosis, *Am. Rev. Tuberc.* 63:427-433, 1951.
11. WAISBREN, B. A., AND SPINK, W. W.: A clinical appraisal of neomycin, *Ann. Int. Med.* 33:1019-1119, 1950.
12. HAWKINS, J. E., JR.: Unpublished observations.
13. AMBERSON, J. B.: A summary of the Second Viomycin Conference. *Tr. 11th Conference on the Chemotherapy of Tuberculosis*, 1952, p. 286-287.

# Kanamycin in Pulmonary Tuberculosis

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Thirty-nine cases of pulmonary tuberculosis on the Pulmonary Disease Service of the Veterans Administration Hospital were treated with kanamycin\* in addition to the previous antituberculous drugs. All cases selected were either moderately advanced or far advanced, with duration of disease varying from 5 to 144 months. To establish some uniformity in the selection of cases, the following requirements were set down: A minimum of four months' previous therapy with two or more antituberculous drugs and either one or both of the following: chest roentgenograms of moderate or far-advanced disease unchanged for at least four months, or continued positive sputa or gastric cultures present for at least four months prior to the start of kanamycin.

In our series of 39 cases, patients ranged in age from 25 to 70 years. According to race, 32 were white and 7 Negro. Twenty-nine cases or 74 per cent were far advanced at the onset of kanamycin treatment, and 10 or 26 per cent were moderately advanced. Thirty-four of the 39 cases (87 per cent) had one or more cavities as shown by roentgenogram. Duration of previous therapy ranged from 4 to 72 months. Twenty-five cases were positive for *Mycobacterium tuberculosis* on either sputa or gastric culture (table I).

Various laboratory procedures including complete blood count, erythrocyte sedimentation rate, urinalysis, audiogram, blood urea nitrogen, fasting blood sugar, serum uric acid, Bromsulphalein, cephalin flocculation, thymol turbidity, and serum albumin and globulin were done before and during therapy to note any possible toxicity. Chest roentgenograms were repeated at two month intervals, and semimonthly sputa or gastric cultures were obtained to determine therapeutic effects.

In the 39 cases selected, all previous medications were continued and intramuscular injections of kanamycin, 1.0 Gm. daily, were given for 28 days. Thereafter the dosage was decreased to 1.0 Gm. three times weekly.

In evaluating the results obtained with kanamycin, it might be well to point out once again that all patients had rather extensive disease of some standing and had had previous antituberculous therapy.

## RESULTS

*Roentgenographic Findings.* Of 19 cases reviewed at the end of two months, 5 cases (26 per cent) showed roentgenologic improvement, and cavity closure confirmed by planography was noted in 3 (16 per cent).

At the end of four months of kanamycin therapy, 17 cases were reviewed. Three or 18 per cent showed improvement roentgenographically, 11 or 65 per cent were unchanged, and 3 (18 per cent) revealed worsening.

*Bacteriological Findings.* Of the 15 cases with positive cultures for *Myco. tuberculosis* before kanamycin therapy, 8 or 53 per cent converted to negative after two months.

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\* The trade name of Bristol Laboratories Inc. for kanamycin is Kantrex.

TABLE I  
*Statistics of 39 Cases before Kanamycin Therapy*

	Number of cases
Age, yr.	
25-35	11
36-45	12
46-60	10
61-70	6
Race	
White	32
Negro	7
Extent of disease	
Moderately advanced	10
Far advanced	29
Known duration of disease before kanamycin, mo.	
4-6	10
6-12	10
12-24	7
24	3
Duration of previous antituberculous therapy, mo.	
4-6	14
6-12	16
12-24	3
24	6
Cavitation present on roentgenography	34
Positive sputa or gastric cultures	25

At the end of four months, 8 (47 per cent) of the 17 patients reviewed had converted to negative (table II).

#### TOXICITY

As regards toxicity to kanamycin, almost all patients complained of a brief transient stinging sensation after injection. Twenty of the 39 patients (50 per cent) complained of intermittent nodule formation at the injection site, usually lasting about three days, but in 10 of these cases one or more nodules persisted longer than three weeks. In 2 cases, treatment had to be discontinued because of multiple persistent nodules, which were moderately painful.

TABLE II  
*Results of Kanamycin Therapy*

	2 months (19 cases)		4 months (17 cases)	
	No. of cases	Per cent	No. of cases	Per cent
Roentgenographic change				
Improvement	5	26	3	18
Cavity closure	3	16		
Unchanged	11	65	11	65
Worse	3	18	3	18
Bacteriological conversion	8 of 15	53	8	47
Urinary findings				
Proteinuria	10	53	11	65
Casts	18	95	17	100

Prior to starting kanamycin, routine laboratory studies in all patients were normal with the following exceptions: Four patients had abnormal cephalin flocculation reactions in 48 hours. Five patients had slight proteinuria, and of these, 3 had glycosuria due to mild diabetes mellitus. In 5 others, occasional fine hyaline or granular casts were noted.

During the administration of kanamycin, there was no increase in any of these abnormalities nor was there any indication of an adverse effect on the 3 diabetic patients.

No toxic effects were noted in the blood count, hemoglobin, blood sugar, Bromsulphalein, thymol turbidity, cephalin flocculation, and albumin-globulin ratio except for transient eosinophilia in some cases. In none of our cases was there an elevation of blood urea nitrogen at any time during the course of therapy.

*Urinary Findings.* During the first two months of kanamycin therapy, repeated urine examinations of 19 patients revealed slight proteinuria in 10 (53 per cent) and occasional hyaline or granular casts in 18 (95 per cent). In some cases casts were noted as early as the first week, but the average time of appearance was from the third to the seventh week after the start of kanamycin.

A review of 17 cases at the end of four months confirmed the occasional occurrence of proteinuria in 11 (65 per cent) and of casts in 17 or 100 per cent of cases.

*Audiographic Findings.* Audiograms taken just prior to the start of this study revealed some varying degrees of abnormality in 23 of the 39 cases (60 per cent). No new damage was demonstrated in the 19 cases reviewed after two months.

After four months of kanamycin, 2 of 12 cases (17 per cent) showed a high tone loss but no previously abnormal audiogram showed any worsening.

#### CONCLUSIONS

1. In a series of patients with pulmonary tuberculosis given kanamycin in addition to the regular antituberculous drugs, favorable roentgenographic and bacteriological changes were noted in a small percentage.
2. Toxic changes due to kanamycin, including transient eosinophilia, intermittent proteinuria and cast formation, mild hearing loss, and formation of nodules at the injection site, have been noted.
3. The use of kanamycin merits consideration as adjunctive therapy in pulmonary tuberculosis, and further study is indicated.

#### ACKNOWLEDGMENTS

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# Kanamycin in Pulmonary Tuberculosis

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Kanamycin\* is a new broad-spectrum antibiotic, which has considerable anti-tuberculous activity in vitro and in experimental animals, as well as little toxicity. Therefore, a clinical investigation was undertaken.

## MATERIAL AND METHOD

Susceptibility of *Mycobacterium tuberculosis* to a single concentration of kanamycin, 10  $\mu\text{g./ml.}$ , was determined in polysorbate 80-albumin medium. Simultaneously, comparative tests with streptomycin, 10  $\mu\text{g./ml.}$ , and isoniazid, 5  $\mu\text{g./ml.}$ , were also run. The inoculum was 0.1 ml. of four to seven day culture in Dubos liquid medium diluted to approximately no. 1 barium sulfate nephelometer. After incubation for 14 days at 37 C., the presence of turbidity in tubes containing drug indicated resistance of the organism, provided Ziehl-Neelsen stained smears of this growth revealed typical acid-fast bacilli. Appropriate growth and media controls were included in all tests.

Eleven patients with pulmonary tuberculosis have had the opportunity to be treated with kanamycin for three to seven months. Kanamycin was administered intramuscularly in a dosage of 1 Gm. once a day to all patients. In 1 patient the dosage was increased at the end of three weeks to 0.5 Gm. three times a day and was continued for five weeks. Adjuvant therapy included isoniazid, 100 mg. three times a day, in 1 patient, which was instituted after two weeks of kanamycin with no response and because the patient was severely ill and was an original treatment case. In addition, 7 patients received sulfisoxazole, 0.5 Gm. four times a day, during kanamycin therapy. This drug was administered because our laboratory has confirmed the Japanese reports of its antituberculous activity in vitro and it was hoped that sulfisoxazole might delay the emergence of resistance to kanamycin. Four patients received kanamycin alone.

The ages of these patients ranged from 31 to 75 years. Seven patients were white and 4 were Negroes. Seven were men. All except 1 had far-advanced disease. One patient had moderately advanced tuberculosis.

The type of disease was chronic in 7 cases and all of these patients were failures on standard antimicrobial therapy. The disease was exudative and toxic in 4 patients; 3 were virgin cases, and 1 had previously received standard therapy.

The diagnosis of tuberculosis was established in 10 of the 11 patients by positive cultures prior to therapy. Their progress was followed with monthly chest films, sputum smears and cultures for tubercle bacilli (except in 1 patient who was psychotic), weekly urinalyses, and weights.

## RESULTS

Twenty-two recently isolated strains of tubercle bacilli were tested for susceptibility to isoniazid, streptomycin, and kanamycin. All strains were susceptible to

\* The trade name of Bristol Laboratories Inc. for kanamycin is Kantrex. Supplies of this drug were generously provided by Bristol Laboratories Inc.

kanamycin, whereas two were resistant to streptomycin and three were resistant to isoniazid. The tubercle bacilli isolated from 7 of the 11 patients prior to therapy with kanamycin were all susceptible to this drug, but one strain was resistant to streptomycin and three strains were resistant to isoniazid.

Although the 11 patients could have been treated with kanamycin for three to seven months, therapy was discontinued after one to five months in 9 patients because of therapeutic failure in 3, toxicity in 4, and both in 2.

In the 4 patients who had exudative toxic disease, defervescence did not occur during the first four weeks of treatment. This is to be contrasted with the fact that standard antimicrobial therapy causes defervescence in more than 80 per cent of previously untreated patients during the first four weeks of treatment.

Sputum conversion on culture can be determined at this time in only 4 patients treated for three months or more. Conversion has failed to occur in these 4 patients. Information on the development of resistance is not yet available.

No significant roentgenographic changes have occurred, with two exceptions. The 1 patient who received isoniazid as well as kanamycin had marked roentgenographic improvement during five months of therapy. This patient had never been treated for tuberculosis before. Another patient, who received kanamycin alone for four months, showed worsening and spread of the disease. He had been a treatment failure on standard therapy.

Weight gain occurred in 6 of the 11 patients, ranging from 0.5 to 7 lb./month. The largest weight gain occurred in the patient who received concurrent isoniazid. Five patients lost weight, ranging from 1 to 3.5 lb./month, including the patient who showed roentgenographic worsening.

Toxicity observations were confined to local reactions and renal and eighth cranial nerve function. Kanamycin was well tolerated by intramuscular injection. Serial routine urinalyses showed more or less constant cylindruria in all but 1 of the patients. Four patients developed albuminuria at two to four months of therapy; 3 of these patients were receiving kanamycin alone. Albuminuria was associated in 2 cases with mild azotemia, which disappeared after therapy was discontinued.

Eighth cranial nerve damage was apparent clinically in 4 patients after one to five months of treatment. This included vestibular disturbance in 1 patient and auditory nerve damage in 3. One of the latter patients became completely deaf at two months of treatment. This severe instance occurred in the patient who weighed only 53 lb. at the start of treatment with 1 Gm./day; she showed no clinical response after three weeks on this dosage, so it was increased to 1.5 Gm./day for five weeks. This is the largest dosage we used, amounting to 62 mg./Kg. a day.

#### SUMMARY

All of 22 strains of tubercle bacilli have been found to be susceptible to kanamycin in a concentration of 10  $\mu$ g./ml. in vitro. Eleven patients with advanced pulmonary tuberculosis, including 7 re-treatment cases and 4 original treatment cases, were given kanamycin, 1 Gm./day. Therapeutic benefit was not demonstrated in terms of temperature response or sputum conversion for the small number of patients for which these values could be measured. Marked roentgenographic improvement occurred in 1 patient who received concurrent isoniazid. Worsening of the disease as seen by roentgenogram occurred in 1 patient treated with kanamycin alone. No change occurred in the rest of the patients. Significant renal and eighth cranial nerve damage resulted in the discontinuation of therapy in 6 of the 11 patients at two to five months of therapy.

# Kanamycin in Genitourinary Infections

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Kanamycin is a water-soluble basic antibiotic that belongs to the neomycin-streptomycin group. Gourevitch et al<sup>1</sup> showed kanamycin to have in vitro and in vivo activity against a wide range of gram-positive, gram-negative, and acid-fast organisms. Dickison and Tisch<sup>2</sup> demonstrated in animals the rapid urinary excretion of the drug, its relative lack of nephrotoxicity as compared with neomycin and vestibular toxicity as compared with streptomycin. Adequate blood levels of kanamycin are readily achieved. In man, following multiple intramuscular injections of kanamycin in the dosage of 0.5 Gm. every six hours, Cronk and Nauman<sup>3</sup> observed average maximum kanamycin serum concentrations of 30  $\mu\text{g.}/\text{ml.}$  and average minimum concentrations of 10  $\mu\text{g.}/\text{ml.}$  Judging from these data, kanamycin appears to be an effective drug for the treatment of genitourinary infections in human beings. The results of a clinical trial are presented.

## PROCEDURE

Thirty-eight patients with genitourinary infections were treated with kanamycin at the Squier Urological Clinic during 1958. There were 34 men and 4 women and their average age was 64 years. Only 2 children were included in this series. The majority of the infections had been treated previously with antibiotic or chemotherapeutic agents and had failed to respond. In this series there were 30 patients who were treated with kanamycin in the immediate period following some surgical procedure on the genitourinary tracts. In those patients who had urinary drainage catheters, an attempt was made to start therapy with kanamycin one or two days before the expected date on which the catheters would be removed.

Immediately before and after kanamycin was administered, a specimen of urine was obtained for bacteriological study. The urines from women were catheterized specimens and the urines from men were either catheterized or clean voided specimens collected in midstream. Blood agar base and E.M.B. media were plated with the urine and brain-heart infusion broth tubes were inoculated. The urine specimen was also incubated and, whenever no growth was obtained from the cultures in 24 hours, the incubated urine was plated again on the media mentioned. When growth was obtained, the gram-negative organisms were identified by utilization of media containing urea, citrate, and sugars, and the gram-positive organisms by colony and microscopic characteristics. The kanamycin sensitivity of the organisms was then determined by the serial tube dilution method.

Kanamycin sulfate was administered intramuscularly in the dosage of 0.5 Gm. every six hours for an average of six days, with a range of 2 to 18 days. One patient received 0.5 Gm. every eight hours, another patient 0.5 Gm. every 12 hours, and another 0.4 Gm. every eight hours.

During kanamycin therapy, all patients were checked daily for tinnitus, vertigo, skin eruption, injection pain, and similar untoward reactions. Daily urinalyses were performed, and complete blood counts and blood urea or nonprotein nitrogen determinations were made three times each week during therapy. Audiograms were performed before and after therapy.

Table I shows the case number, unit number, sex, age, clinical diagnosis, dose

TABLE I  
The Effect of Kanamycin on 38 Patients with Genitourinary Infections

Case no.	Unit no.	Age Sex	Diagnosis	Treatment (total dose)	Culture before treatment	Culture after treatment
1	1364350	74 M	Carcinoma prostate	(0.5 x 4) x 7 (12 Gm.)	<i>Alcaligenes faecalis</i> <i>E. coli</i>	<i>Pseudomonas</i>
2	148439	67 M	Carcinoma prostate	(0.5 x 4) x 6 (11 Gm.)	<i>A. aerogenes</i>	No growth
3	976247	77 M	Carcinoma bladder (catheter throughout treatment)	(0.5 x 4) x 7 (12 Gm.)	<i>A. aerogenes</i>	No growth
4	253890	75 M	Benign prostatic hypertrophy	(0.5 x 4) x 6 (11 Gm.)	<i>A. aerogenes</i>	No growth
5	1376804	83 M	Benign prostatic hypertrophy, bladder calculus	(0.5 x 4) x 12 (24.5 Gm.)	<i>A. aerogenes</i>	No growth
6	1374591	82 M	Benign prostatic hypertrophy	(0.5 x 4) x 6 (10.5 Gm.)	<i>A. aerogenes</i> <i>B. proteus</i>	<i>Pseudomonas</i>
7	848837	62 M	Carcinoma kidney; residual urine = 90 ml.	(0.5 x 4) x 9 (17 Gm.)	<i>B. proteus</i>	<i>Pseudomonas</i>
8	1381676	63 M	Benign prostatic hypertrophy	(0.5 x 4) x 6 (10.5 Gm.)	<i>Pseudomonas</i>	No growth
9	983043	58 F	Chronic pyelonephritis, right, with hydronephrosis and calculi	(0.5 x 4) x 7 (12 Gm.)	<i>B. proteus</i>	No growth
10	1383942	32 M	Acute pyelonephritis right, obstructing ureteral calculus	(0.5 x 4) x 9 (16 Gm.)	<i>E. coli</i>	Enterococcus
11	1339124	77 M	Benign prostatic hypertrophy, bilateral hydronephrosis, hypotonic bladder, (catheter throughout treatment)	(0.5 x 4) x 10 (18.5 Gm.)	<i>A. aerogenes</i> <i>Pseudomonas</i>	Enterococcus
12	1346588	83 F	Carcinoma bladder (catheter throughout treatment)	(0.5 x 4) x 6 (10 Gm.)	<i>Pseudomonas</i> Enterococcus	<i>Pseudomonas</i> Enterococcus
13	971638	58 M	Benign prostatic hypertrophy	(0.5 x 4) x 12 (24 Gm.)	<i>B. proteus</i>	No growth
14	067256	13 F	Chronic cystitis, neurogenic bladder, residual urine = 75 ml.	(0.5 x 4) x 5 (8.5 Gm.)	<i>E. coli</i>	<i>Pseudomonas</i>
15	1350087	64 M	Chronic cystitis, neurogenic bladder, residual urine = 75 ml.	(0.5 x 4) x 7 (12 Gm.)	<i>E. coli</i>	No growth
16	1369286	78 M	Carcinoma prostate	(0.5 x 3) x 4 (3.5 Gm.)	<i>A. aerogenes</i>	Enterococcus
17	084973	8 M	Benign prostatic hypertrophy	(0.5 x 4) x 13 (25 Gm.)	<i>A. aerogenes</i>	No growth
18	1380554	61 M	Acute pyelonephritis, solitary kidney, obstructive oliguria septicemia	(0.4 x 3) x 18 (19.2 Gm., 60 mg./Kg./day)	<i>A. aerogenes</i> (urine) <i>A. aerogenes</i> (blood)	No growth (urine) No growth (blood)
19	1371933	77 M	Benign prostatic hypertrophy (catheter throughout treatment)	(0.5 x 4) x 9 (16.5 Gm.)	<i>A. aerogenes</i> <i>Pseudomonas</i>	No growth
20	1380639	84 M	Benign prostatic hypertrophy	(0.5 x 4) x 5 (8.5 Gm.)	<i>A. aerogenes</i>	No growth
21	1384312	58 M	Scrotal abscess, chronic prostatitis with calculi	(0.5 x 4) x 7 (13 Gm.)	<i>M. pyogenes</i> var. <i>aureus</i>	<i>Pseudomonas</i>
22	1371747	54 M	Carcinoma prostate	(0.5 x 4) x 9 (15.5 Gm.)	Enterococcus (urine) <i>E. coli</i> (scrotum)	No growth (urine) <i>Pseudomonas</i> (scrotum)
23	098930	66 M	Benign prostatic hypertrophy	(0.5 x 4) x 6 (9.5 Gm.)	<i>B. proteus</i> <i>Pseudomonas</i>	<i>B. proteus</i>
24	1357583	24 M	Periurethral abscess (catheter throughout treatment)	(0.5 x 4) x 7 (11 Gm.)	<i>Pseudomonas</i>	<i>Pseudomonas</i>
25	1374968	81 M	Benign prostatic hypertrophy	(0.5 x 4) x 11 (19 Gm.)	<i>M. pyogenes</i> var. <i>aureus</i>	No growth
				(0.5 x 4) x 5 (7.5 Gm.)	<i>A. aerogenes</i>	Enterococcus <i>C. albicans</i>

Table I Continued on Page 715

TABLE I (Continued)

*The Effect of Kanamycin on 38 Patients with Genitourinary Infections*

Case no.	Unit no.	Age Sex	Diagnosis	Treatment (total dose)	Culture before treatment	Culture after treatment
26	1383223	85 M	Carcinoma penis	(0.5 x 4) x 6 (10 Gm.)	<i>B. proteus</i>	No growth
27	202861	70 M	Carcinoma kidney pelvis, nephrosclerosis (catheter throughout treatment)	(0.5 x 4) x 4 (6 Gm.)	<i>B. proteus</i>	<i>B. proteus</i> <i>Pseudomonas</i>
28	1358845	83 M	Benign prostatic hypertrophy	(0.5 x 4) x 6 (11 Gm.)	<i>A. aerogenes</i>	No report
29	1371327	33 F	Nephroptosis, left, with hydronephrosis	(0.5 x 4) x 7 (12 Gm.)	No growth	No growth
30	971908	74 M	Benign prostatic hypertrophy	(0.5 x 4) x 3 (4.5 Gm.)	No growth	No growth
31	837018	63 M	Carcinoma prostate	(0.5 x 4) x 4 (6 Gm.)	<i>E. coli</i>	No report
32	1374723	77 M	Benign prostatic hypertrophy, bilateral hydronephrosis	(0.5 x 4) x 1 (2 Gm.)	<i>A. aerogenes</i>	No report
33	1273144	45 M	Acute pyelonephritis, right with obstructing ureteral calculus	(0.5 x 4) x 4 (6.5 Gm.)	<i>A. aerogenes</i>	No report
34	1378736	45 M	Benign prostatic hypertrophy	(0.5 x 4) x 8 (13 Gm.)	No growth	No growth
35	918998	72 M	Benign prostatic hypertrophy	(0.5 x 4) x 5 (5.5 Gm.)	No growth	No report
36	1380332	60 M	Benign prostatic hypertrophy	(0.5 x 4) x 5 (9.5 Gm.)	<i>E. coli</i>	No report
37	1317213	70 M	Carcinoma prostate	(0.5 x 4) x 8 (14.5 Gm.)	No growth	No growth
38	1129320	78 M	Benign prostatic hypertrophy, bladder calculus	(0.5 x 2) x 5 (5 Gm.)	No report	No report

of kanamycin, culture of urine before therapy and culture of urine after therapy in the 38 patients treated with kanamycin. There were 27 patients (cases 1 to 27) who had positive cultures before therapy and in whom adequate culture data following therapy were available. The remaining 11 patients (cases 28 to 38) included 5 patients whose urine cultures before therapy revealed no growth and 6 patients whose urine cultures before and/or after therapy were not reported at the time of data analysis. All of the 5 patients whose urine cultures before therapy revealed no growth were treated during the immediate postoperative period when urinary drainage tubes were in place, and the urine cultures of all 5 patients continued to reveal no growth at the termination of therapy, by which time the catheters had been removed. The group of 27 patients comprised 29 infections of which 28 were of the urinary tract. One patient was treated twice for urinary infection and one patient had a scrotal abscess that was treated immediately following surgical incision and drainage.

## RESULTS

Following therapy, pyuria cleared and the urine culture was sterile in 15 of the 29 genitourinary infections. The culture following treatment of the remaining 14 infections revealed the persistence of the same organisms, which were isolated before therapy and/or the presence of new organisms, infection with which was acquired during treatment. Four of the 29 infections were acute in the sense of elevated temperature and clinical evidence of an acute process and in 2 infection was absent at the end of therapy. Seven urinary infections were complicated by the

TABLE II

*Effect of Kanamycin on 29 Genitourinary Infections According to Organisms Isolated before and after Therapy*

Organisms	Number isolated before therapy			Number isolated after therapy	
	Total	Eradicated	Persisted	New organisms	Total
<i>A. aerogenes</i>	12	12	0	0	0
<i>B. proteus</i>	7	5	2	0	2
<i>Pseudomonas</i>	6	4	2	7	9
<i>E. coli</i>	5	5	0	0	0
Enterococcus	2	1	1	4	5
<i>M. pyogenes</i> var. <i>aureus</i>	2	2	0	0	0
<i>A. faecalis</i>	1	1	0	0	0
<i>Candida albicans</i>	0	0	0	1	1
Total	35	30	5	12	17

presence of urinary drainage tubes throughout the entire course of kanamycin therapy and in 4 infection was absent at the end of treatment. In 2 patients with lower urinary tract infections there was elevated bladder residual urine throughout therapy and in 1 infection was absent at the end of therapy. Two chronic urinary tract infections were complicated by the presence of calculous disease and in 1 infection was absent at the end of therapy. One acute urinary tract infection with complicating calculous disease persisted following treatment.

Table II shows the effect of kanamycin on the organisms isolated before and after treatment of the 29 infections. Thirty-five organisms were isolated before and 17 after therapy. *Aerobacter aerogenes* was eradicated in all 12 instances in which it was isolated prior to therapy and *A. aerogenes* did not appear as a new organism following treatment. Similarly, all of the 5 *Escherichia coli* and the 2 *Micrococcus pyogenes* var. *aureus* infections were eradicated during therapy, and neither *E. coli* nor *M. pyogenes* var. *aureus* appeared as new organisms following therapy. On the other hand, only 5 of 7 *Bacillus proteus* infections, 4 of 6 *Pseudomonas*, and 1 of 2 enterococci were eradicated. In addition, *Pseudomonas* appeared in 7 instances as new organisms following therapy, enterococci in 4 instances and *Candida albicans* in 1 instance following therapy.

Kanamycin sensitivities were performed on 38 organisms that were isolated from 27 of the 38 patients with genitourinary infections. Table III compares the kanamycin sensitivities of the organisms with the effect of treatment of the infections with kanamycin. In general there was close correlation between the kanamycin sensitivity and the response of the organism to treatment. All 4 organisms that persisted after therapy were resistant to concentrations of kanamycin less than 25  $\mu\text{g./ml.}$  However, 1 *A. aerogenes* and 2 *E. coli* infections were eradicated during treatment despite their resistance to kanamycin in concentrations less than 50  $\mu\text{g./ml.}$  Three of 4 eradicated *Pseudomonas* infections were resistant to kanamycin concentrations less than 25  $\mu\text{g./ml.}$ , and 2 *B. proteus* organisms were eradicated although the minimum inhibitory concentrations of kanamycin were 25 and 100  $\mu\text{g./ml.}$  The eradication of these relatively resistant organisms is explained at least in part by the high dosage of kanamycin used in this series of patients.

#### TOLERANCE

Kanamycin was well tolerated by the majority of the 38 patients.

Kanamycin was administered to 3 patients who had significant gross impairment

TABLE III

Kanamycin Sensitivity of 38 Organisms Isolated before and after Kanamycin Therapy

Organisms isolated	Number isolated before therapy ( $\mu\text{g./ml. kanamycin}^*$ )		No culture report after therapy	Number isolated as new organisms after therapy ( $\mu\text{g./ml. kana-}$ $\text{mycin}^*$ )
	Eradicated	Persisted		
<i>A. aerogenes</i>	11 (0.78-6.25) 1 (50)	0	2 (3.12, 6.25)	0
<i>Pseudomonas</i>	1 (6.25) 2 (25, 25) 1 (100)	2 (50, >100)	0	7 (50->100)
<i>E. coli</i>	2 (0.78, 12.5) 2 (50, 50)	0	2 (0.39, 12.5)	0
<i>B. proteus</i>	2 (25, 100)	1 (25)	0	0
<i>Enterococcus</i>	0	1 (>100)	0	0
<i>M. pyogenes</i> var. <i>aureus</i>	1 (1.56)	0	0	0
Total	23	4	4	7

\* Minimum inhibitory concentration of kanamycin.

of total renal function before therapy and to 4 patients who had significant gross functional impairment of one kidney before therapy. All but 1 patient showed some evidence of additional renal toxicity during treatment. In general the changes were minimal and did not necessitate cessation of kanamycin therapy. Table IV shows the case number, age, diagnosis, dose of kanamycin, blood urea or non-protein nitrogen levels on hospital admission and before therapy, and the renal

TABLE IV

Renal Toxicity in 7 Patients with Poor Renal Function During Kanamycin Therapy

Case number	Age	Diagnosis	Dose, Gm.	Admission blood urea nitrogen (nonprotein nitrogen), mg%	Blood urea nitrogen (nonprotein nitrogen) before therapy, mg%	Toxicity during therapy
<i>Bilateral Impairment</i>						
11	77	Benign prostatic hypertrophy, bilateral hydronephrosis	28.5 (2 courses)	30	19	None
17	8	Acute pyelonephritis in solitary kidney, obstructive oliguria, septicemia	19.2	(35)	(125)	Casts appeared, persisted
37	70	Carcinoma prostate	14.5	54	54	Blood urea nitrogen increased (76, then 62 mg./100 ml.)
<i>Unilateral Impairment</i>						
9	58	Right chronic pyelonephritis with hydronephrosis, pelvic calculi	12.0	19	19	Blood urea nitrogen increased (29, then 21 mg./100 ml.)
10	32	Right ureteral obstruction with stone, acute pyelonephritis	16.0	27	24	Casts appeared at end of therapy
29	33	Left nephroptosis with hydronephrosis	12.0	18	18	Casts appeared at end of therapy
33	45	Right ureteral obstruction with stone, acute pyelonephritis	6.5	23	20	Casts increased at end of therapy

toxicity, which was observed during treatment. In 1 patient (case 27) the blood urea level, which was elevated before therapy, rose significantly during treatment without the appearance of casts or change in albuminuria. The blood urea level in this patient did not return to the pretreatment level until three days following the cessation of treatment. On the other hand, one patient (case 17) showed marked improvement of renal function during therapy despite severe impairment of total renal function when therapy was initiated. This 8 year old boy, chronically ill with Cooley's anemia, had acute pyelonephritis caused by *A. aerogenes* in a solitary kidney. Acute inflammatory ureteral obstruction resulted in oliguria which was relieved 24 hours after onset by ureteral catheter drainage. However, *A. aerogenes* septicemia had already occurred. Cultures of blood and urine continued to show *A. aerogenes* when kanamycin therapy was started four days after the onset of the acute infection. At this time the patient was critically ill, the blood nonprotein nitrogen was 125 mg./100 ml. and the carbon dioxide combining power was 7.9 mEq./l. Within two days following the initiation of treatment with kanamycin in the dosage 60 mg./Kg./day cultures of the blood and urine were sterile, and within five days the blood nonprotein nitrogen fell to 25 mg./100 ml. Cylindruria appeared on the tenth day following the start of therapy and was the only evidence of additional renal toxicity during treatment.

Some evidence of renal toxicity was noted in 19 of the 38 patients in the series. Twelve of the 19 patients were treated immediately following urinary tract surgery and all of the remaining patients had instrumentation of the urinary tract before or during therapy. Microscopic hematuria therefore could not be used as a criterion of renal toxicity. Urinary albumen was qualitatively increased at least 2 plus units in 2 patients during therapy. Cylindruria appeared or was qualitatively increased in 14 patients during treatment. In 4 patients casts appeared during therapy then disappeared before the end of therapy. In general, hyaline casts appeared first and were followed by granular casts. On the other hand, the cylindruria of 1 patient before therapy persisted unchanged during therapy and in 2 patients the cylindruria which was present before therapy disappeared during therapy. Blood urea nitrogen levels rose in 6 patients during therapy. In 4 patients who had normal levels before therapy, the levels during therapy were minimally elevated (27 to 31 mg./100 ml.) and returned to normal levels before the end of therapy. On the other hand, the blood urea nitrogen levels, which were elevated in 2 patients before therapy, showed falls to normal levels during therapy. In those patients who before therapy had no signs of impaired renal function by the screening tests employed, 11 Gm. of kanamycin was the minimum dose which was associated with some evidence of renal toxicity. In those patients who had signs of abnormal renal function prior to therapy, 4.5 Gm. of kanamycin was the minimum dose which was associated with evidence of additional impairment of renal function.

Audiometric tests were performed both before and after treatment with kanamycin in 24 of the 38 patients in this series. The remaining 14 patients had no subjective evidence of disturbance of the auditory apparatus, except for one patient who had abnormal audiometric and caloric tests following therapy without control tests prior to therapy. This patient (case 17) received 19.2 Gm. of kanamycin in the dosage 60 mg./Kg./day for 18 days. The patient complained of decreased hearing in the left ear without tinnitus three days following cessation of treatment. The audiogram thereafter revealed on the left a loss of 50 to 65 decibels air conduction in all frequencies and on the right a loss of 65 decibels air conduction only at 8000 cycles/second. Although vertigo was absent, the caloric test which was

TABLE V  
*Hearing Loss in 5 Patients with Audiograms Performed before and after  
Therapy with Kanamycin*

Case number	Age	Dose, Gm.	Audiometric deficit before therapy Air conduction				Additional audiometric deficit after therapy Air conduction				Symptoms
			Right		Left		Right		Left		
			Decibels	c/sec	Decibels	c/sec	Decibels	c/sec	Decibels	c/sec	
2	67	11.0	−40	2000	−40	2000	−20	all	−20	all	None
5	83	24.5	−60	4000	−60	4000	−10	all	−10	all	Moderate hearing loss
			−65	8000	−65	8000					
			−40	125 to 4000	−40	125 to 2000					
			−70	8000	−30	4000					
					−75	8000					
8	63	10.5	−60	8000	−45	8000	−10	8000	None		None
16	78	25.0	−35	4000	−35	4000	−10	8000	−10	8000	None
			−65	8000	−65	8000					
36	60	9.5	−15	4000	−15	4000	None		−20	8000	None

done at the time hearing loss appeared revealed mild hypofunction of the left labyrinth. Audiometric and caloric tests, which were performed one month later, continued to show the same changes.

Audiometric changes following therapy were observed in 5 of the 24 patients who had audiograms performed before therapy. Table V shows the case number, age, dose of kanamycin, audiometric deficit before therapy, additional audiometric deficit following therapy and the symptoms experienced in these 5 patients. All of the 5 patients were beyond middle age and all had at least some bilateral audiometric deficit before therapy. Following treatment, 3 patients had additional audiometric deficits only at 8000 cycles/second, and in 2 patients this additional deficit was unilateral. In the remaining 2 patients there was significant bilateral additional deficit in all frequencies. Minimal audiogram changes occurred in the 3 patients who had minimal hearing impairment before therapy, irrespective of the dose of kanamycin. However, in 1 patient (case 5) who had significant hearing loss prior to therapy and high dose of kanamycin, there was additional impairment in all frequencies bilaterally following therapy. Subjective hearing loss occurred in this patient without tinnitus on the day kanamycin was discontinued. An audiogram, which was performed on this patient one month later, showed no change over that performed at the termination of therapy. Subjective hearing loss or tinnitus did not occur in the other 4 patients and follow-up audiograms were not available. One of the 5 patients experienced vertigo attacks following therapy. However, the attacks had occurred previously and had not increased in frequency after therapy. In the group of 6 patients in whom audiometric change or hearing loss were evident following treatment, there were 3 patients who also showed some evidence of renal toxicity during therapy.

The total white and red cell counts and hemoglobin levels of the 38 patients during kanamycin therapy showed no changes that were attributable to the drug. However, the differential white cell counts performed during therapy revealed mild eosinophilia (range 5 to 7 per cent) in 8 patients whose eosinophil concentrations were previously normal. In 4 of the 8 patients the eosinophilia disappeared before the termination of therapy. One patient, before therapy, had mild eosinophilia, which persisted at the same level during and following therapy, and another patient had mild eosinophilia, which disappeared during therapy. No patient de-

veloped skin eruption or other allergic manifestation during treatment with kanamycin. The eosinophilia occurred along with some evidence of renal toxicity in 4 patients and with change in auditory nerve function in 2 patients.

The intramuscular injection of kanamycin in the concentration 0.5 Gm./ml. was associated with mild, transient burning discomfort at the site of injection in all patients and, in the majority, was of the same order of pain intensity as that which follows the injection of penicillin. However, 3 of the 38 patients who received the drug experienced injection pain that lasted from one to six hours and therapy in one of these patients had to be discontinued for this reason. The intensity of discomfort in 2 patients was minimized by the addition of 0.5 ml. of 1 per cent procaine to the dose of kanamycin.

#### SUMMARY

Thirty-eight patients with genitourinary infections were treated with kanamycin sulfate in the dosage of 0.5 Gm. intramuscularly every six hours for an average of six days.

Twenty-nine genitourinary infections with cultural data adequate for analysis were treated, with the following results: (1) Fifty per cent of all the infections cleared entirely; (2) all 12 infections with *Aerobacter aerogenes* were eradicated as were all 5 with *Escherichia coli* and 2 with *Micrococcus pyrogenes* var. *aureus*; (3) five out of 7 *B. proteus* infections were eradicated; (4) *Pseudomonas* and enterococci infections were little affected by the treatment and these organisms frequently appeared following therapy when not present previously; (5) the kanamycin sensitivities of the organisms generally predicted correctly the response of the infections to treatment.

Drug toxicity was studied in the 38 patients with the following results: (1) Seven patients, with bilateral or unilateral impairment of renal function before therapy, showed little additional impairment during therapy. One patient with severe renal damage before therapy showed marked improvement in renal function during therapy. The data indicate that pre-existing renal damage is not necessarily a contraindication for kanamycin therapy; (2) 50 per cent of the patients showed evidence of mild renal irritation. In none of the patients was it severe enough to warrant cessation of therapy; (3) 16 per cent of the patients suffered hearing loss by audiogram. The data suggest that auditory toxicity may occur more frequently in those patients who already have some hearing deficit and that the severity of toxicity is greater when the original hearing loss is significant; (4) mild eosinophilia occurred in 20 per cent of the patients without other allergic manifestation; (5) 3 patients complained of significant injection pain.

#### ACKNOWLEDGMENT

The kanamycin used in this study was generously supplied by Bristol Laboratories Inc., Syracuse, New York.

#### BIBLIOGRAPHY

1. GOUREVITCH, A.; HUNT, G. A., AND LEIN, J.: Antibacterial activity of kanamycin, *Antib. & Chemo.* 8:149, March, 1958.
2. DICKSON, H. L., AND TISCH, D. E.: A preliminary report on the pharmacology of kanamycin. Transactions of the 17th Conference on the Chemotherapy of Tuberculosis.
3. CRONK, G. A., AND NAUMANN, D. E.: Clinical and laboratory studies with kanamycin, *Ann. New York Acad. Sc.* 76:308, 1958.

# Treatment of the Typhoid Carrier State with Kanamycin

## A Preliminary Report

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In spite of the fact that typhoid fever is no longer a major problem, the presence of chronic typhoid carriers in the general population is a continuing threat to the public health. The Illinois State Department of Public Health had 300 registered known carriers as of September 19, 1958. This is not a large number, but, in the presence of favorable environmental circumstances, any one of these few carriers might trigger an epidemic. For this reason, they are kept under surveillance by the health authorities, at some expense to the health budget and at the loss of much of the carrier's personal freedom.

In special populations, e.g., patients in mental hospitals, the hazard created by the chronic typhoid carrier is brought into sharp focus. The control of typhoid carriers depends largely on each carrier's ability to comprehend the danger he presents to others and on his willingness to manage his life and affairs so that the risk to others can be minimized. If the carrier is psychotic, the danger is magnified, because he may be incapable of either understanding the problem or of taking appropriate precautions. Therefore, typhoid carriers in mental hospitals have to be segregated from other patients so that the necessary sanitary precautions can be systematically undertaken for them.

At the present time, there are 30 carriers in the state mental hospitals in Illinois. Most of these carriers are the residual of the now historic typhoid outbreak at Manteno State Hospital, in 1939. These carriers are segregated in a special building, housing no other patients, where they receive special nursing care and attention. Although 30 is not a large number, the building might otherwise be used for as many as 150 patients. Hence, they give rise to a net deficit of 120 hospital beds within the state mental hospital system. Thus, 120 badly needed beds could be added to the system if these 30 carriers could be cured.

Attempts to accomplish this by surgical and medical means have been made for many years, particularly following the sudden increment of 110 carriers that resulted from the Manteno outbreak in 1939. Cholecystectomy was performed in approximately 60 per cent of the patients. Antibacterial and antibiotic therapies have been systematically employed, as each new drug was introduced. These therapeutic agents included iodophthalein, many sulfonamides, penicillin, chloramphenicol, carbomycin, and synnematin B. None of these antibacterial agents was effective. The remaining 30 typhoid carriers represent the residual after subtraction of those carriers who were either cured by cholecystectomy, eliminated by death, or discharged from the institution into the care of relatives after the signing of a typhoid carrier agreement with the state health department.

Kanamycin sulfate\* is the most recent antibiotic systematically investigated for this purpose. In vitro studies by Gourevitch and associates have shown that kanamycin is active against *Salmonella*.<sup>1</sup> Clinical studies by Thurman and Platou indicated that it is clinically effective in many *Salmonella* infections.<sup>2</sup> Their studies in

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\* The trade name of Bristol Laboratories Inc. for kanamycin sulfate is Kantrex.

the treatment of typhoid fever were encouraging but not conclusive, because of the few patients available for study. While kanamycin has some toxic potential, this was not sufficiently prominent to contraindicate its use for this purpose. This paper is a preliminary report of the method and results of these clinical trials.

#### MATERIALS AND METHODS

All of the known chronic typhoid carriers now residing in mental hospitals maintained by the Illinois Department of Public Welfare were included in the kanamycin study. The 30 carriers are segregated in one building at the Manteno State Hospital. No other mental patients are housed in this building. Within the building, separate facilities are provided for men and women.

Of the men, 5 are white and 2 are Negroes, with ages ranging from 35 to 84 years old. Psychiatric diagnoses of the men included dementia praecox, chronic brain syndrome due to neurosyphilis or arteriosclerosis, senile dementia, cerebral arteriosclerosis, and mental deficiency. Four of the patients were in good contact and cooperative; 3 were confused and in poor contact but fairly cooperative. The patients exhibited a variety of somatic diseases (arteriosclerotic heart disease, hypertensive heart disease, tuberculosis, and carcinoma of the skin), but all were considered to be in satisfactory physical condition at the time of the pretreatment physical examination. Stool specimens were collected routinely at monthly intervals after admission as a typhoid carrier. All of the 7 carriers had at least two positive stool cultures during the two year period preceding kanamycin treatment. It is well known that typhoid carriers vary in the constancy with which they shed typhoid bacilli. Two of the men were considered "persistent," i.e., they had three or less negative stool specimens during the two years preceding the present study. The remaining 5 "intermittent" carriers had three or more negative stools during the preceding two years.

The group of women consisted of 20 whites and 3 Negroes, ranging in age from 50 to 85 years old. Psychiatric diagnoses included the usual cross section found in similar hospital groups: dementia praecox, general paresis, senile dementia, chronic brain syndrome, and mental deficiency. Most of the women were quiet, although many were very confused and in poor contact. Many had mild to moderately severe somatic diseases, e.g., hypertension, arteriosclerotic heart disease, or bronchial asthma, but all were considered to be in satisfactory physical condition at the time of pretreatment physical examination. Twelve women were persistent carriers and 11 were intermittent carriers.

During kanamycin treatment, the following clinical and laboratory observations were made: pulse, temperature and blood pressure determinations, urinalysis, non-protein nitrogen, complete blood counts, Bromsulphalein tests, and subjective evaluation for possible hearing loss.

Post-treatment stool specimens were collected for bacteriological examinations on a twice weekly schedule. During the initial phase of the study, specimens were split between the state approved laboratory at Manteno State Hospital and the branch laboratory of the Illinois State Health Department. During later phases of the study, specimens were collected at weekly intervals and examined at Manteno State Hospital laboratory only.

The study is being carried out in three phases, differing slightly as to dosage schedule:

*Phase 1.* Beginning on June 27, 1958, 7 male typhoid carriers received 2.0 Gm./

TABLE I  
*Bacteriological Follow-up in 5 Typhoid Carriers Treated with Kanamycin*

No. of weeks, post-treatment, before stool became positive	No. of carriers	
	At end of period	Accumulated
2.5	2	2
3.0	1	3
4.0	0	3
5.0	0	3
6.0	3	6

day kanamycin sulfate, given intramuscularly in two divided doses, plus 1.5 Gm./day, given orally in three divided doses for 14 days. Total intramuscular dose of kanamycin was 28 Gm. per carrier.

*Phase 2.* Beginning on August 28, 1958, the same group of carriers was re-treated, this time with 3.0 Gm./day kanamycin sulfate, given intramuscularly in two divided doses, plus 1.5 Gm./day, given orally in three divided doses. Total dose of intramuscular kanamycin was 42 Gm. per carrier. In addition, each patient received 25 mg. of cortisone per day.

*Phase 3.* Beginning on September 2, 1958, the group of 23 women carriers received 3.0 Gm./day kanamycin sulfate, given intramuscularly, plus 1.5 Gm./day, given orally. Total dose of intramuscular kanamycin administered was 42 Gm. per carrier.

## RESULTS

*Phase 1.* Bacteriological follow-up began three days after kanamycin treatment stopped. The results are shown in table I.

One carrier remained negative at the time he was re-treated. This patient, an intermittent carrier, had only two positive stools during the preceding year.

*Phase 2.* Bacteriological follow-up began on the third post-treatment day and will be continued: five of seven specimens were all negative. The remaining two specimens were collected 12 days after treatment. One was positive and one negative. Fourteen days after treatment, follow-up specimens were collected from all carriers. In 5 carriers the results were negative; in the 2 persistent carriers, the results were positive.

*Phase 3.* Bacteriological follow-up began on the third post-treatment day in 14 carriers. All 14 specimens were negative. Follow-up specimens, collected from the 23 carriers 11 days after treatment, were negative in 18 carriers and positive in 5.

## DISCUSSION

The only untoward effects resulting from the administration of kanamycin consisted of transient albuminuria in 6 cases, slight elevation of temperature in 5, and nausea and vomiting in 3. These side effects disappeared promptly after cessation of therapy.

The normal intestinal flora of coliform bacteria and enterococcal streptococci appeared to have been almost completely wiped out during therapy. No overgrowth of yeast or *Staphylococcus aureus* was found. For this reason, it was considered wise to administer a multivitamin preparation after conclusion of the treatment regimen.

## CONCLUSIONS

Kanamycin is the most promising drug we have studied so far for the relief of the typhoid carrier state. While the dosage schedule used (28 or 42 Gm. given intramuscularly plus 21 Gm. given orally for 14 days) did not cure the typhoid carrier state, the high degree of antibacterial activity of kanamycin was indicated by the fact that even persistent carriers had negative stools for two to three weeks after cessation of treatment. These findings point to four important aspects: (1) the post-treatment follow-up of carriers must be systematic and prolonged before concluding that any typhoid carrier is "cured"; (2) kanamycin, even in relatively large dosage, is virtually nontoxic and well tolerated in the type of patients studied; (3) further study is necessary, using other kanamycin dosage regimens, and, perhaps, other drugs in combination with kanamycin (a study using cortisone and kanamycin is now under way); and (4) the possibility of using kanamycin on a maintenance dosage schedule to control carriers should be investigated.

## ACKNOWLEDGMENT

Kanamycin for these studies was generously supplied by Bristol Laboratories Inc.

## BIBLIOGRAPHY

1. GOUREVITCH, A.; HUNT, G. A., AND LEIN, J.: Antibacterial activity of kanamycin, *Antib. & Chemo.* 8:149, March, 1958.
2. THURMAN, W. G. AND PLATOU, R. V.: Some experiences with kanamycin in the treatment of *Salmonella* and *Shigella* infections, *Ann. New York Acad. Sc.* 76:230, 1958.

# Treatment of Chronic Amebiasis, Brucellosis, and Other Infections with Kanamycin

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Our previous studies<sup>1,2</sup> have revealed that kanamycin\* is effective in the treatment of anthrax, staphylococcal infections, and urinary tract infections caused by *Proteus* species. It was further found that the drug was doubtfully active in brucellosis, typhoid, and *Pseudomonas* infections.

In the present report, observations will be described that confirm many of those previously made, and, in addition, data will be presented that show that kanamycin caused a rapid disappearance of cysts of *Entamoeba histolytica* from the stools of asymptomatic carriers. More limited studies also suggest that it is effective in the treatment of acute amebic colitis.

## CLINICAL MATERIAL

*Amebiasis.* The patients, asymptomatic carriers of *E. histolytica*, were largely residents at an orphanage in Guadalajara. The institution was known to have a high incidence of amebic infection, and, in June, 1958, single specimens from an unselected group of 83 boys and girls revealed a carrier rate of *E. histolytica* cysts of 70 per cent. In the study, kanamycin was given in doses of 30, 50, and 150 mg./Kg. body weight/day for a period of 10 days to separate groups of carriers. Another previously unreported group, treated a few months earlier with a mixture of methylanilide and chloroquin,† was included in this study for comparison with kanamycin. These patients received six capsules (methylanilide, 2.7 Gm., and chloroquin, 0.3 Gm.) each day for ten days. Details concerning the treatment groups are shown in table I.

*Bacterial Infections.* Patients with bacterial infections treated with kanamycin were studied at Vanderbilt Hospital and the Veterans Administration Hospital, Nashville, or the Civil Hospital in Guadalajara. Several patients with brucellosis also received novobiocin. The patient with typhoid also received chloramphenicol.

## METHODS

*Bacteriological.* Common pathogenic bacteria were isolated and identified by conventional laboratory procedures. Blood cultures for *Brucella* were made in Castaneda bottles<sup>3</sup> in Tryptose phosphate broth and agar without added carbon dioxide gas. Bottles were incubated for 15 days before discarding as negative. Assays for kanamycin in urine, cerebrospinal fluid, and serum were performed by a twofold serial dilution test with test volumes of 1 ml. in Wassermann tubes. All tests were per-

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This study was supported by a grant from Bristol Laboratories Inc., Syracuse, N. Y.

\* The trade name of Bristol Laboratories Inc. for kanamycin is Kantrex.

† Methylanilide, 450 mg., and chloroquin, 50 mg., in capsules supplied by The Upjohn Company, Kalamazoo, Mich.

TABLE I

*Description of Carriers\* of Entamoeba histolytica Cysts Treated with Kanamycin*

Age distribution, yr.	Dosage kanamycin, mg./Kg.			Methylanilide-chloroquin
	150	50	30	
<5	1	3	—	—
5-10	14	12	7	—
11-20	3	7	8	16
>21	1	—	—	4
Total	19	22	15	20

\* Sixty-seven of the 76 patients were residents at the orphanage, and the remaining 9 were patients at the Civil Hospital in Guadalajara.

formed in heart infusion broth, using a  $10^1$  dilution of an overnight growth of culture as inoculum. The standard culture was a recently isolated strain of *Staphylococcus albus* inhibited by  $0.25 \mu\text{g.}/\text{ml.}$  of kanamycin.

*Parasitological.* Fresh stool specimens were examined microscopically, following the zinc sulfate flotation and concentration methods of Faust et al.<sup>4</sup>

## RESULTS

*Asymptomatic Carriers.* In figure 1 and table II may be seen the results of treatment of carriers of amebic cysts with three different regimens of kanamycin and with the methylanilide-chloroquin mixture. Included for comparison are results of treatment with oxytetracycline of a similar group of 65 children with amebic cysts.<sup>5</sup> It will be seen that cysts disappeared most rapidly from the stools of children receiving oxytetracycline and least rapidly from those treated with methylanilide-chloroquin mixture. Cysts disappeared from the stools of patients receiving kanamycin at an intermediate rate. Of great interest was the close coincidence of the disappearance rates, with all three regimens of kanamycin, including dosages that varied fivefold. With the two higher doses of kanamycin and with methylanilide-chloroquin treatment, 2 or 3 patients continued to discharge cysts. One patient, a 17 year old girl, was in both the 150 mg. kanamycin and the methylanilide-chloroquin group. She was subsequently treated with tetracycline, and the cysts disappeared. However, it

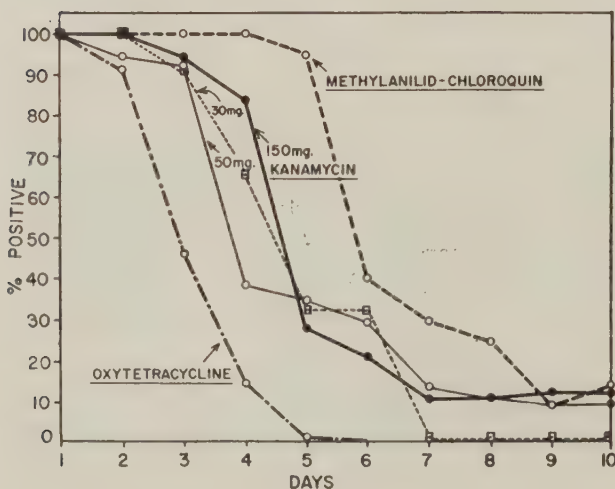
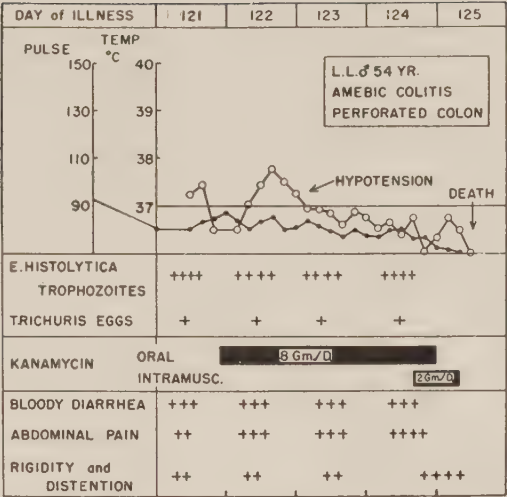


FIG. 1. Cysts disappeared at nearly the same rate from patients treated with widely varying doses of kanamycin. These curves were intermediate between those of patients treated with oxytetracycline and those receiving a mixture of methylanilide and chloroquin.

FIG. 2. This 54 year old man died of peritonitis following perforation of an amebic ulcer of the upper sigmoid colon despite four days treatment with kanamycin.



has not been an unusual experience to encounter an occasional patient with amebic cysts who resists treatment with most of the available antiamebic drugs.

Observation of these patients after treatment revealed that about one-fourth of the patients treated with oxytetracycline once again were discharging cysts in each of three consecutive monthly follow-up examinations, and, in about one third of the cases treated with methylanilide-chloroquin, four and five months after treatment. One month after treatment with 150 mg./Kg. of kanamycin, 8 of 14 patients examined were again discharging cysts.

*Amebic Colitis.* In table III may be seen the results of treatment of 3 cases of amebic colitis. Two of moderate severity in small children responded promptly, with cessation of bloody diarrhea and clearing of trophozoites from the stools within three or four days. A third patient, a 54 year old man (fig. 2), had been ill for at least 121 days and survived only four days during treatment with kanamycin. His course was steadily downhill, characterized by severe tenesmus, bloody diarrhea, abdominal pain, and, finally, severe hypotension. At autopsy he was found to have a large perforated amebic ulcer at the upper end of the sigmoid colon and general

TABLE II  
Results of Treatment of Asymptomatic Carriers of Cysts of *Entamoeba histolytica*

Day	Kanamycin therapy, mg./Kg. (no. patients)						Methylanilide-chloroquin (20 patients)		Oxytetracycline <sup>a</sup> (65 patients)
	150 (19)		50 (22)		30 (15)		No. patients examined	Per cent positive	
	No. patients examined	Per cent positive	No. patients examined	Per cent positive	No. patients examined	Per cent positive			
1	19	100	22	100	15	100	20	100	100
2	19	100	18	94	15	100	20	100	92
3	18	94	14	93	15	93	20	100	46
4	19	84	18	39	15	66	20	100	15
5	18	28	17	35	15	33	20	95	2
6	18	22	20	30	15	33	20	40	0
7	18	11	21	14	15	0	20	30	
8	18	11	17	11	15	0	20	25	
9	17	12	20	10	15	0	20	10	
10	17	12	21	14	15	0	20	10	

<sup>a</sup> See reference 4.

TABLE III  
Clinical Summary of Patients Treated with Kanamycin

Age, yr.	Sex	Hospital*	Diagnosis	Previous treatment	Kanamycin			Response	Comment
					Gm./day	Route	No. days		
1	M	HC	Amebic colitis, moderately severe	None	2	Oral	10	Improved	Trophozoites persisted three days, cleared on fourth; cysts disappeared on seventh day; diarrhea reduced from 12 to 3/day; blood in stool cleared by fifth day
2	M	HC	Amebic colitis, moderately severe	None	2	Oral	10	Improved	Trophozoites disappeared after two days; asymptomatic by this time
54	M	HC	Amebic colitis with perforation, severe	Several antibiotics and antiamebics	2 8	Oral Intramuscular	4 1	None	Ill 121 days before treatment; critically ill when first treated; death from perforation of colon on fourth day; trophozoites, gross blood in stool until death (fig. 2)
40	F	HC	Acute bacteremic brucellosis	None	2	Intramuscular	10	None	Four months illness with fever; blood culture positive on fourth day of kanamycin; novobiocin five days later without effect; prompt cure with tetracycline (fig. 3)
15	M	HC	Acute bacteremic brucellosis	None	2	Intramuscular	8	None	Continued fever and painful enlarged liver during treatment; blood culture positive after eight days of kanamycin; novobiocin for five days, associated with slow improvement (fig. 4)
39	F	HC	Acute bacteremic brucellosis	None	2	Intramuscular	7	None	Nearly continuous illness for seven months, with fever, back pain, weakness; no clinical response to kanamycin; several blood cultures positive during first five days of treatment; slow or doubtful response to later novobiocin (fig. 5)
14	F	HC	Acute bacteremic brucellosis	None	2	Intramuscular	7	None	Ill 46 days; blood culture positive fifth day of kanamycin; later slow recovery during novobiocin
31	M	HC	Acute bacteremic brucellosis	None	2	Intramuscular	11	None	Ill several weeks before kanamycin; two blood cultures positive before treatment; low fever persisted during five days of treatment
18	F	HC	Typhoid	None	2	Intramuscular	8	None	Temperature increased during kanamycin; relieved by intravenous chloramphenicol (fig. 6)

Table III Continued on Page 729

TABLE III (Continued)  
Clinical Summary of Patients Treated with Kanamycin

Age, yr.	Sex	Hospital <sup>a</sup>	Diagnosis	Previous treatment	Kanamycin			Response	Comment
					Gm./day	Route	No. days		
23	M	HC	Staphylococcal pyoderma, acute, right hand and forearm	None	2	Intramuscular	9	Excellent	Very rapid improvement with treatment
22	F	HC	Breast abscess, severe	None	2	Intramuscular	11	Good	Temperature of 40.1 C. at admission lysed to normal in seven days; on tenth day, green pus from nipple with <i>Ps. aeruginosa</i> and recurrent fever (considered to represent complication of kanamycin treatment)
28	M	VH	Empyema ( <i>Ps. aeruginosa</i> )	Chloramphenicol, 2 Gm./day, for 10 days; penicillin, 10 days	2	Intramuscular	7	None	Infection later controlled with local instillation polymyxin B
33	M	HC	Gonorrhea	None	2	Intramuscular	8	Slight	Smear and culture positive after four days treatment; first negative on sixth day; relapse with positive smear and culture twelfth day; treated with penicillin
53	M	HC	Paracolon <i>Bacillus</i> septicemia	None	2	Intramuscular	5	Slight	Alcoholic found in coma; defervescence with fluid and kanamycin; bacteremia cleared by third day, later high fever, hypotension, and pericardial friction followed by death (fig. 7)
29	M	VH	Aortic endarteritis traumatic (paracolon <i>Bacillus</i> )	Chloramphenicol and penicillin, 10 days	2	Intramuscular	4	None	Infection followed chest wound; improved following 4 Gm. chloramphenicol and 3 Gm. streptomycin for four days and later lower dose
39	F	VH	Paralytic poliomyelitis, chronic bilateral pyelonephritis ( <i>Proteus</i> sp.)	Many previous treatments for four years	2	Intramuscular	10	Excellent	No negative cultures in four years until kanamycin; pyuria disappeared
79	M	VA	Chronic bilateral pyelonephritis ( <i>Proteus</i> sp.)	Chloramphenicol, 2 Gm./day	2	Intramuscular	10	Excellent	Bacteruria and pyuria cleared in five days; became afebrile and greatly improved
32	M	VA	Acute bilateral pyelonephritis ( <i>E. coli</i> )	None	2	Intramuscular	7	Excellent	Became afebrile; cultures negative; pyuria cleared in six days
2	M	VH	Hydrocephalus, meningitis ( <i>A. aerogenes</i> )	Chloramphenicol, 50 mg./Kg. for 10 days, intramuscularly	30†	Intramuscular	6	None	Finally improved with high doses of chloramphenicol and tetracycline

<sup>a</sup> HC, Hospital Civil, Guadalajara; VH, Vanderbilt Hospital, Nashville; and VA, Veterans Administration Hospital, Nashville.

† This dosage is given in mg./Kg. body weight/day.

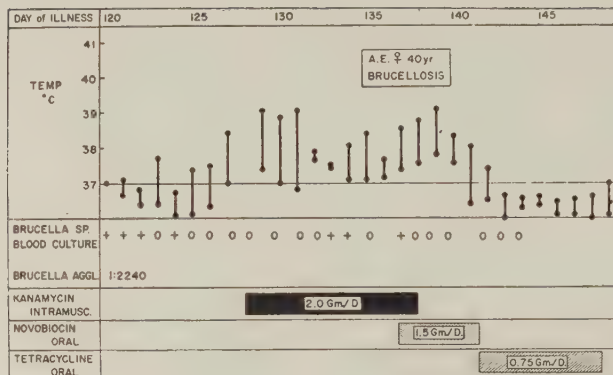


FIG. 3. This 40 year old woman with brucellosis failed to respond to kanamycin as evidenced by continued fever and bacteremia. She did not improve with novobiocin but recovered promptly with tetracycline.

peritonitis. In retrospect, it was considered that the perforation had occurred one or two days before death. There was no evidence of benefit by treatment with kanamycin, either by the oral or intramuscular route. His stools also constantly contained numerous ova of *Trichuris trichiura*.

**Acute Brucellosis with Bacteremia.** Five patients with this disease were treated with kanamycin (table III), and all had positive blood cultures. There was no clinical evidence of benefit from kanamycin, and blood cultures were positive 5, 5, 8, and 10 days, respectively after the start of treatment with kanamycin in 4 of the cases. The fifth patient, although bacteremic before treatment, was less severely ill than the other 4. An example of the course of one of the patients is shown in figure 3. This patient, a 40 year old housewife, had been ill for three months. During the first 10 days of kanamycin treatment, several blood cultures were positive for *Brucella* sp., and her clinical condition became worse. She was then given novobiocin orally, and although there was slow lysis of fever, she continued to be quite ill. Only after a subsequent course of tetracycline did she become well.

The second patient, a 15 year old boy, failed to improve on kanamycin and had several positive blood cultures during the first eight days of treatment (fig. 4). His illness was characterized by an enlarged tender liver, which became worse during kanamycin treatment. During subsequent treatment with novobiocin, no more blood cultures were positive and his temperature gradually returned to normal, but the liver tenderness and enlargement persisted for several days after he was afebrile. Novobiocin treatment was terminated prematurely because of a generalized morbilliform rash, which cleared after the drug was stopped. He subsequently became asymptomatic and was discharged.

The course of a third patient with brucellosis treated with kanamycin is shown in

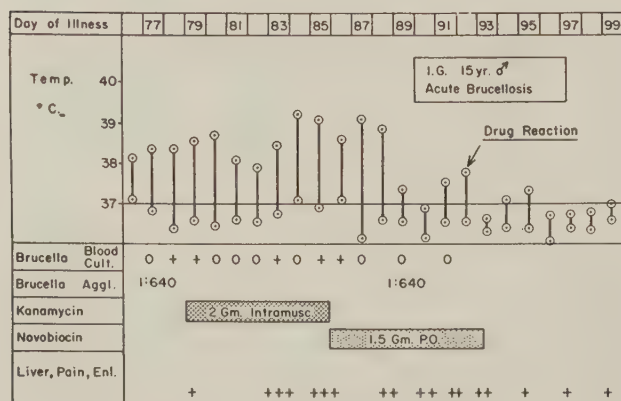


FIG. 4. This 15 year old boy with acute brucellosis failed to respond to kanamycin. Slow recovery occurred with novobiocin treatment, which was later complicated by a drug eruption.

FIG. 5. This 39 year old woman with brucellosis did not respond to kanamycin. Slow defervescence and recovery occurred during treatment with novobiocin.

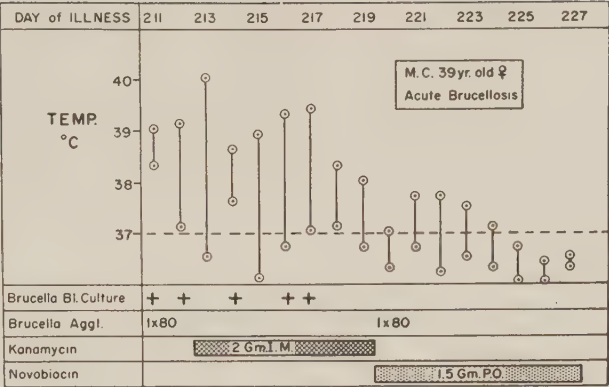


figure 5. This 39 year old woman continued to have positive blood cultures for several days. When shifted to novobiocin she gradually improved.

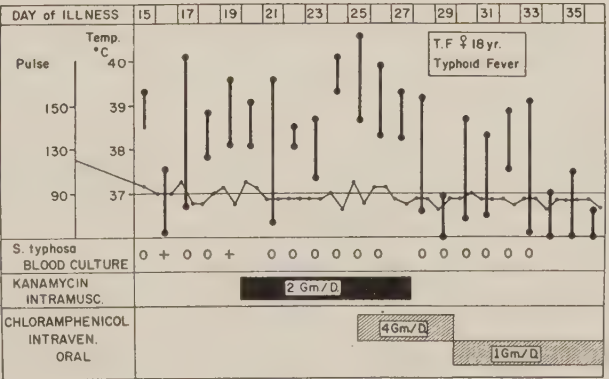
**Typhoid.** One patient with typhoid fever (table III) was treated without apparent response. Her course of illness is shown in figure 6. Blood cultures were only positive before kanamycin treatment, but fever and prostration persisted for seven days, during which the temperature became higher. Because of this, chloramphenicol was given intravenously, followed by defervescence and great symptomatic improvement on the third day. When the dose was lowered to 1 Gm. daily given orally, however, small afternoon elevations of temperature recurred for several days without symptoms. Recovery thereafter was complete.

**Staphylococcal Infection.** One patient with staphylococcal pyoderma and another with breast infection presumed to be staphylococcal responded promptly to treatment with kanamycin (table III). The patient with breast infection had a relapse during the second week when cultures of green pus from her nipple yielded *Pseudomonas aeruginosa*. This infection did not respond to continued kanamycin treatment, but later, after incision and drainage, she recovered.

In addition to the preceding case, in which *Pseudomonas* infection apparently developed during treatment with kanamycin, a second patient with an empyema with this organism failed to respond to kanamycin (table III). He did improve later following local instillations of polymyxin B.

**Gonorrhea.** One patient with gonorrhea, a 33 year old male, failed to respond to eight days of treatment with 2 Gm. daily of kanamycin given intramuscularly. Cultures and smears revealed gonococci during each of the first four days of treatment, but with diminishing urethral discharge. Cultures and smears on the seventh and eighth days were negative. Treatment was stopped at this time. He was asymptoma-

FIG. 6. This patient with typhoid fever became worse during nine days of treatment with kanamycin. Treatment with chloramphenicol, 4 Gm. daily, intramuscularly, was associated with defervescence and remission in three days. Afternoon fever returned for four days without symptoms when the dose was reduced to 1 Gm. daily.



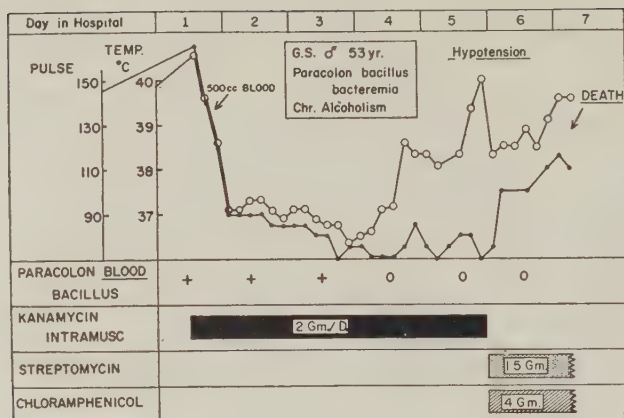


FIG. 7. This chronic alcoholic male with paracolon bacillus bacteremia responded to kanamycin and supportive treatment with clinical improvement and disappearance of bacteremia. Later myocardial weakness and hypotension appeared which resulted in death.

tic until the twelfth day in the hospital, when he once again developed urethral discharge containing gonococci. His infection was then successfully treated with penicillin.

**Paracolon Bacillus Bacteremia.** Two patients, severely ill with paracolon *Bacillus* bacteremia complicating other diseases, were treated (table III). In 1, a 53 year old chronic alcoholic (fig. 7), clinical improvement occurred following rehydration and kanamycin given intramuscularly, and the blood culture became negative on the fourth day. Shortly thereafter, however, he abruptly developed hypotension, a pericardial friction rub, and myocardial weakness. He failed to improve with various therapies and died in a state of cardiovascular collapse. The other patient with endarteritis of traumatic origin showed no evidence of response to treatment with kanamycin but improved later with chloramphenicol and streptomycin.

**Pyelonephritis.** Two patients with severe pyelonephritis caused by *Proteus* sp. responded well to treatment with kanamycin (table III). One was a 39 year old woman with severe paralytic poliomyelitis of five years' duration, who required constant respiratory aid. She had had *Proteus* organisms in all urine cultures in recent years, despite treatment with a wide variety of antimicrobial drugs. With kanamycin, however, cultures became negative, and she improved sufficiently to leave the hospital. Another patient with acute pyelonephritis caused by *Escherichia coli* responded well to treatment with kanamycin (table III).

**Meningitis.** A patient with hydrocephalus and meningeal infection with *Aerobacter aerogenes* failed to respond to kanamycin given intramuscularly but finally improved with high doses of chloramphenicol and tetracycline.

#### KANAMYCIN IN SERUM AND CEREBROSPINAL FLUID

Nine pairs of serum and cerebrospinal fluid samples from 6 different patients, collected at various time intervals after 1 Gm. doses given intramuscularly every 12 hours over a period of five days, were assayed for kanamycin. The serum specimens showed concentrations of kanamycin as follows: 2, less than 0.5  $\mu\text{g./ml.}$ ; 1, 2.0  $\mu\text{g./ml.}$ ; 2, 4.0  $\mu\text{g./ml.}$ ; 2, 8.0  $\mu\text{g./ml.}$ ; and 2, 16.0  $\mu\text{g./ml.}$  Only one cerebrospinal fluid specimen contained assayable kanamycin (0.5  $\mu\text{g./ml.}$ ); all others were negative ( $<0.5 \mu\text{g./ml.}$ ).

#### KANAMYCIN IN BLOOD AND URINE

The absorption of kanamycin after oral administration was studied in 6 adult

patients receiving 8 Gm. daily in four equally divided doses. Among 24 serum specimens obtained at intervals distributed over nine days of treatment, 17 had values that were not measurable, one was 0.5  $\mu\text{g./ml.}$ , four were 1.0  $\mu\text{g./ml.}$ , and two were 2.0  $\mu\text{g./ml.}$  There was no indication that serum concentrations increased with continued treatment. Seventeen specimens of urine, paired with blood specimens taken during the first five days, were assayed. No drug was detected in 4, 5 had 32  $\mu\text{g./ml.}$ , 2 had 64, 3 had 128, 1 had 200, and 2 had greater than 256  $\mu\text{g./ml.}$  Some urine specimens contained measurable kanamycin in each of the five days. Positive urine values were generally associated with positive serum values, but there was no suggestion of accumulation of drug.

Two additional adult patients were given 2 Gm. of kanamycin orally every six hours, and blood specimens for assay were collected at 1, 3, 6, 24, 25, 27, and 30 hours. In 1 patient the only measurable values were 1.0, 2.0, and 2.0  $\mu\text{g./ml.}$  at 24, 25, and 27 hours, respectively. In the other, the only measurable values were 1.0, 0.5, 0.5, and 1.0  $\mu\text{g./ml.}$  at 6, 25, 27, and 30 hours, respectively.

#### TOXICITY

During the study, no deafness or other neurotoxicity was observed, and no instances of abnormal renal function attributable to drug treatment were detected. The only untoward effect of the drug was transient mild diarrhea with bulky yellow stools in 9 of 19 children receiving 150 mg./Kg. body weight/day of kanamycin. These manifestations appeared on the third to the ninth days of treatment, but in each case treatment was continued for a full 10 day period without undue distress. No diarrhea was observed with the smaller doses of kanamycin.

#### DISCUSSION

These studies have disclosed a substantially beneficial effect of kanamycin in the treatment of asymptomatic carriers of amebic cysts. The activity appears to be somewhat less than that of oxytetracycline but greater than that of the methylanilide-chloroquin mixture. The most interesting observation in connection with this part of the study was the virtually identical effect of three widely varying doses of the drug. It appears that the factor of therapy responsible for eliminating amebic cysts reaches an optimal effect at a low dosage. This question will be pursued in further studies.

Observations a long period after treatment of the patients who received kanamycin are not yet available. About one-half of the group who received 150 mg./Kg. have again become positive. This is a higher rate of recurrence than that with the patients who received oxytetracycline and the methylanilide-chloroquin mixture. While this may indicate less effective therapy with kanamycin, the obvious opportunity for easy re-infection in that environment makes us hesitant to interpret these results too specifically at present.

Neomycin, chemically similar to kanamycin, is also active against amebae. Most reports describe its use in conjunction with other drugs, and, in those in which it has been used alone, the effect has not been impressive.<sup>6</sup> From those studies and the present data, it is our impression that kanamycin is more active than neomycin, but the paucity of reports with the use of neomycin alone does not provide an adequate basis for a final evaluation.

Our experience with acute amebic colitis is small. The 2 moderately ill patients

recovered promptly and, we believe, received definite therapeutic benefit from kanamycin. The patient who died was critically ill, and it seemed doubtful that he would have responded to any treatment. One of us has observed fatality in amebiasis with perforation of the bowel in patients treated with tetracycline and oxytetracycline, indicating the enormous therapeutic challenge of patients severely ill with amebiasis.

Based on the studies in this report and those previously made,<sup>1,2</sup> it is apparent that kanamycin is active in anthrax, staphylococcal infection, and, to a considerable extent, in urinary tract infections due to *Proteus* sp. There was some evidence, based on return to negative of blood cultures, that kanamycin was also effective in paracolon *Bacillus* infection. It is also definite that the agent alone is not active in brucellosis, typhoid, and *Ps. aeruginosa* infection. The failure of the drug to be effective in a case of meningitis with *A. aerogenes* may be related to its inadequate penetration into the cerebrospinal fluid, rather than poor antimicrobial effect.

In this study, 5 patients acutely ill with brucellosis were given novobiocin after several days of apparently ineffective treatment with kanamycin. In this situation, with spontaneous remission an increasing possibility, evaluation of drug effect was admittedly difficult. It can be said that slow recovery characterized the course of patients receiving novobiocin, and none had positive blood cultures after this drug was started. Based on previous experience in this area<sup>7-9</sup> in the treatment of brucellosis with the tetracyclines and chloramphenicol, it is our impression that, if novobiocin possesses activity in relieving the acute manifestations of brucellosis, it must be appreciably less than that of the tetracyclines and chloramphenicol. An adequate answer to the question will depend upon treatment of more patients.

The observation that kanamycin is absorbed to only a slight degree after several days treatment by the oral route is in agreement with other reports.<sup>10</sup> Furthermore, the negligible extent of penetration of this agent into the cerebrospinal fluid after intramuscular administration suggests that it may only be used effectively in meningitis when administered by the intrathecal route.

#### SUMMARY

Kanamycin was found to be effective in the treatment of 51 of 56 cases of asymptomatic carriers of *Entamoeba histolytica* cysts. Doses ranging from 30 to 150 mg./Kg./day showed the same degree of activity. Another regimen, a mixture of methylanilide and chloroquin, was active, but less so than kanamycin.

Two of 3 patients with acute amebic colitis responded well to kanamycin. Kanamycin, when used alone, was found to be ineffective in acute brucellosis and was apparently without effect in typhoid or gonorrhea. It was considered beneficial in 2 cases of staphylococcal infection, 2 of *Proteus* infection, and 1 with *Escherichia coli* but was without activity in 2 cases of *Pseudomonas* infection.

The drug was very slightly absorbed after large, continued oral doses, and spinal fluid assays were almost uniformly negative during several days of treatment with 2 Gm./day given intramuscularly.

#### BIBLIOGRAPHY

1. RUIZ SANCHEZ, F., AND RUIZ SANCHEZ, A.: Laboratory and clinical experiences with kanamycin, Ann. New York Acad. Sc. 76:235-241, 1958.
2. WHITE, A. C., AND KNIGHT, V.: Kanamycin: pharmacologic, microbiologic and clinical observations, Ann. New York Acad. Sc. 76:277-288, 1958.
3. CASTANEDA, M. R.: A practical method for routine blood cultures in brucellosis, Proc. Soc. Exper. Biol. & Med. 64:114, 1947.

4. FAUST, E. C.; D'ANTONI, J. S.; ODOM, V.; MILLER, M. J.; PERES, C.; SAWITZ, W.; THOMEN, L. F.; TOBIE, J., AND WALKER, J. H.: A critical study of clinical laboratory techniques for the diagnosis of protozoan cysts and helminth eggs in feces. I. Preliminary communication, *Am. J. Trop. Med.* 18:169, 1938.
5. RUIZ SANCHEZ, F.; RUIZ SANCHEZ, A.; LUIS CHAM, J., AND NARANJO GRANDA, E.: Treatment of chronic amebiasis with oxytetracycline and tetracycline, *AM & CT* 4:460-464, 1957.
6. NETER, E.: Infantile diarrheal disease and enteric infections. *In*: Waksman, S. A., Ed.: Neomycin, its nature and practical application, Baltimore, Williams and Wilkins Co., 1958.
7. KNIGHT, V.; RUIZ SANCHEZ, F.; RUIZ SANCHEZ, A., AND McDERMOTT, W.: Aureomycin in typhus and brucellosis, *Am. J. Med.* 4:407, 1949.
8. KNIGHT, V.; RUIZ SANCHEZ, F., AND McDERMOTT, W.: Chloramphenicol in the treatment of the acute manifestations of brucellosis, *Am. J. Med. Sc.* 219:627-638, 1950.
9. KNIGHT, V.; RUIZ SANCHEZ, F., AND RUIZ SANCHEZ, A.: Terramycin in treatment of human brucellosis, *A.M.A. Arch. Int. Med.* 87:835-843, 1951.
10. TISCH, D. E.; HUFTALEN, J. B., AND DICKISON, H. L.: Pharmacological studies with kanamycin, *Ann. New York Acad. Sc.* 76:44-65, 1958.

# The Current Status of Kanamycin Therapy

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The rising incidence of serious infections caused by organisms resistant to the commonly used antibiotics has led to the search for agents effective against such infections. One such antibiotic discovered recently is kanamycin, isolated from *Streptomyces kanamyceticus* by Umezawa at the Japanese National Institute of Health and Tokyo University.<sup>1,2</sup>

The original in vitro and in vivo studies in Japan demonstrated that kanamycin was active against many gram-negative pathogens, against strains of *Staphylococcus* resistant to other antibiotics, and against streptomycin- and isoniazid-resistant strains of *Mycobacterium tuberculosis*. Clinical studies were carried out initially on cases of pulmonary tuberculosis, but subsequent clinical reports have shown the drug to be effective against infections due to a wide range of microorganisms.

At a recent conference on kanamycin held at the New York Academy of Sciences in July, 1958, the pharmacological studies and clinical experiences on the use of the drug to date were presented. Cron and his co-workers<sup>4</sup> determined the chemical composition of kanamycin and showed that hydrolysis will yield 2 amino sugars linked glycosidically to 2-dioxystreptamine. The drug is water-soluble, stable through a pH of 2 to 11 under normal sterilizing conditions and at a boiling temperature for 30 minutes at a pH of 6 to 8. The structural formula resembles that of neomycin, in that there is a common dioxystreptamine moiety, but the two antibiotics differ in the amino sugars.

Toxicity studies on laboratory animals have shown the acute toxicity to be of a relatively low degree.<sup>3</sup> Toxicity produced by subacute or chronic administration of kanamycin was evident on the vestibular and auditory functions of the experimentally treated animals, similar to that produced by streptomycin and dihydrostreptomycin, although much larger doses and longer periods of administration were required to produce similar effects. Renal damage has been produced in dogs by prolonged kanamycin administration, but this has been considerably less than that produced by neomycin and required larger doses administered for longer periods.

Microbiological studies on kanamycin have revealed a wide range of in vitro activity against the *Staphylococcus*, the *Bacillus* group, *Vibrio*, *Salmonella*, *Shigella*, *Mycobacterium*, and some strains of *Proteus* and *Pseudomonas*. The antibiotic is relatively inactive against most streptococci, pneumococci, and most anaerobic organisms<sup>5</sup> (see table I). The antibacterial action of kanamycin is primarily bactericidal.

Kanamycin exhibits complete cross resistance to neomycin and partial cross resistance to streptomycin; that is, all neomycin-resistant strains of organisms are kanamycin-resistant and neomycin-sensitive strains are kanamycin-sensitive, but some streptomycin-resistant strains are kanamycin-sensitive. Gourevitch and co-workers,<sup>5</sup> using serial transfer techniques on *Staphylococcus aureus* and *Escherichia coli*, have demonstrated the development of resistance to kanamycin in vitro in a slow, stepwise fashion. Such resulting resistant strains follow the pattern of complete cross resistance to neomycin and incomplete cross resistance to streptomycin.

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TABLE I  
*In Vitro Sensitivities of Various Microorganisms to Kanamycin*

Organisms usually sensitive	Organisms occasionally sensitive	Organisms usually resistant
Staphylococci	<i>Proteus</i>	Pneumococcus
<i>E. coli</i>	<i>Pseudomonas</i>	<i>Streptococcus</i>
<i>Aerobacter-Klebsiella</i>		Yeast
<i>B. anthracis</i>		Fungi
<i>N. gonorrhoeae</i>		
<i>Salmonella</i>		Anaerobic microorganisms
<i>Shigella</i>		

No cross resistance with any of the other antibiotics has been consistently demonstrated.

Following intramuscular administration, kanamycin is absorbed rapidly and excreted in the urine by glomerular filtration. Peak serum concentrations are obtained in one hour, and about 80 per cent of the drug is eliminated in the urine within 24 hours. Doses of 0.25 to 0.5 Gm. give serum concentrations that inhibit the most sensitive organisms, and serum levels after doses of 1.0 Gm. will inhibit the less sensitive strains. To maintain effective drug levels in the blood, the drug has to be administered at intervals of from 6 to 12 hours.<sup>6,7</sup> The pharmacologic properties of kanamycin are as follows: (1) Absorbed rapidly after intramuscular administration. Extremely poor absorption from the gastrointestinal tract. (2) Primarily excreted into the urine by glomerular filtration. (3) Freely soluble in water but insoluble in the common alcohols. (4) Stable throughout a pH range of 2 to 11 under normal sterilizing conditions and at boiling temperatures for 30 minutes at pH 6 to 8.

The majority of cases of resistant staphylococcal infections, including some cases of staphylococcal endocarditis, have been reported to respond favorably to kanamycin therapy. Dosages used in adults have been from 1 to 3 Gm. daily and in infants from 12.5 to 50 mg./Kg. body weight/day (see table II). Urinary tract infections due to sensitive organisms, most cases of gonorrhea, and anthrax were all reported to show good responses.<sup>6,8-14</sup> *Salmonella* and *Shigella* infections have been treated with both the oral and intramuscular forms of kanamycin with good results.<sup>15</sup> Response in brucellosis, pneumococcal and streptococcal infections, and typhoid fever, were reported to be not particularly favorable.<sup>8</sup> Kanamycin therapy of tuberculosis due to strains resistant to other agents has been encouraging, but kanamycin-resistant organisms have emerged within 3 to 4 months in some cases when used alone<sup>16-18</sup> (table III).

Kanamycin has been administered orally in doses of 4 to 8 Gm. daily as a means of reducing fecal flora prior to bowel surgery.<sup>19,20</sup> Aerobic fecal organisms are elimi-

TABLE II  
*Dosage Recommendations for Kanamycin*

Intramuscular	Oral	Local
Adult	4 to 8 Gm. daily (for sterilization of bowel)	2.5 mg./ml.
1 Gm. every 8 hours in severe cases		
0.5 Gm. every 6 hours in moderately severe cases		
0.5 Gm. every 8 or 12 hours in mild cases		
Children		
50 mg./Kg. body wt./day in severe cases		
25 mg./Kg. body wt./day in moderately severe cases		
12.5 mg./Kg. body wt./day in mild cases		

TABLE III

*Responses of Kanamycin to Various Infections*

Infections that have responded favorably to kanamycin	Infections that have not usually responded to kanamycin
Staphylococcal infections	Pneumococcal infections
Furunculosis	Streptococcal infections
Septicemia	Typhoid fever
Endocarditis	Brucellosis
Osteomyelitis	Most cases of infection due to <i>Pseudomonas</i>
Infections due to <i>E. coli</i>	Some cases of <i>Proteus</i> infections
Urinary tract infection	Infections caused by yeast, fungi and anaerobic organisms
Infection due to <i>Aerobacter-Klebsiella</i>	
Urinary tract infection	
Pneumonias	
Most cases of <i>Proteus</i> infections	
Anthrax	
Gonorrhea	
Salmonellosis	
Shigellosis	

nated, but anaerobic organisms usually persist. Reduction in the blood ammonia level during kanamycin administration has been observed with beneficial effect on the clinical and electroencephalographic signs of liver failure.<sup>20,21</sup> Serum levels of the drug following oral administration are quite low, in the range of 2 to 8  $\mu\text{g.}/\text{ml.}$  Kanamycin has also been used for intraperitoneal instillation in cases of contaminated or infected peritoneal cavity with favorable results.<sup>22</sup> The optimum local concentration appears to be in the range of 0.2 to 0.5 per cent, but higher concentrations have been instilled locally into wounds. Kanamycin has been administered by slow intravenous drip in a few cases. Local thrombophlebitis does not appear to be a problem.

The major toxic effects reported from the use of kanamycin have been those involving the eighth nerve and the kidney. Urinary casts have been the most commonly reported abnormality, although there have been a few cases of albuminuria, elevated nonprotein nitrogen, and even oliguria reported.<sup>6,7,12,14</sup> Preliminary reports on renal function tests done in patients receiving the drug suggest a slight decrease in glomerular filtration rate and tubular function in about a third of the cases.<sup>23</sup> Acute tubular necrosis accompanied clinically by oliguria and a rise in the blood urea nitrogen associated with the use of kanamycin has been reported.<sup>24</sup> The patient in this case had a mild elevation of the blood urea nitrogen at the start of therapy and received a total dosage of 21 Gm. administered over a period of two weeks. Evidence of auditory nerve damage has been demonstrated by audiometric studies in patients receiving high dosages of kanamycin for periods of two weeks or longer. The deafness that develops appears and reaches its maximum during therapy, and, from follow-up studies, is usually but not always bilateral, complete, or permanent. Tinnitus is frequently a warning sign of auditory nerve damage. The clinical studies thus far suggest that significant ototoxicity begins after administration of 30 to 40 Gm., but in patients with glomerular damage toxic effects may appear earlier.<sup>6</sup> Mild skin rashes have been observed during kanamycin therapy, but these have been quite rare and may not, in fact, have been due to kanamycin. There has been no evidence of bone marrow suppression or liver injury reported, but some patients have exhibited an increase in eosinophils in the peripheral blood.

During the past nine months approximately 150 patients receiving kanamycin

therapy have been studied in the Texas Medical Center in Houston, Texas. Observations on the first 100 of these patients were presented at the New York Academy of Science meeting in July.<sup>13,14</sup> Approximately 50 of these patients in the study were a part of a staphylococcal epidemic occurring in the newborn nursery at Jefferson Davis Hospital. Kanamycin therapy had little effect on the incidence of infections during this epidemic but had a marked effect on morbidity and mortality. Almost all of the patients treated in the early part of the study in Houston had serious infections, and kanamycin was usually given after unsuccessful therapy with a variety of antibiotics. More recently, kanamycin has been used more extensively on the basis of laboratory evidence of resistance of the causative organisms to other antibiotics, because the need for an actively bactericidal antibiotic was obvious, or because intramuscular route of administration was preferable in patients with infections resistant to other antibiotics with intramuscular preparations.

Most of the adult patients treated with kanamycin had other systemic diseases, such as advanced generative lesions of the cardiovascular, renal, and respiratory systems, and disorders of the hematopoietic system.

Associated local lesions were found in a few of the patients, and parenteral administration of kanamycin was supplemented by local instillation and the institution of surgical drainage. Simple focal lesions, such as furunculosis unassociated with systemic reactions, were not treated with kanamycin.

Dosages used in adult patients ranged from 1 to 3 Gm. daily in divided doses at intervals of 6 to 12 hours. Infants received doses of 12.5 to 50 mg./Kg. body weight/day. These dosage differences were dependent on severity of the illness being treated. Kanamycin was also instilled into body and abscess cavities in concentrations of 2.5 mg./ml.

The sensitivity of the organisms isolated from the patients were determined by the standard disc technique and, in most of the cases, by the tube dilution method, as described by Gourevitch.<sup>5</sup> The antibacterial action of kanamycin was found to be predominantly bactericidal, the concentration producing a bactericidal effect being the same as the bacteriostatic concentration or the next higher concentration.

Most of the strains of staphylococci isolated were resistant to penicillin, streptomycin, and tetracycline by the standard disc and plate dilution sensitivity methods. All of these strains were found to be sensitive to neomycin, novobiocin, ristocetin, and vancomycin (see table IV). Tube dilution sensitivity tests performed on 67 strains of staphylococci revealed inhibition of multiplication in all cases by concentrations of 6.12  $\mu\text{g./ml.}$  or less. The bactericidal concentration was found to be 6.25  $\mu\text{g./ml.}$  or less in all but one strain.

Similar in vitro studies done on *Escherichia coli* showed inhibition of growth of 15 strains by a concentration of 12.5  $\mu\text{g./ml.}$  All of these strains were also inhibited by neomycin. Five strains of the *Aerobacter-Klebsiella* group studied had sensitivities in the same range as that of the strains of *E. coli*.

Most of the strains of *Proteus* isolated were inhibited by concentrations of 25  $\mu\text{g./ml.}$  or less. Five strains each of *Pseudomonas* and streptococci studied were resistant to concentrations of 12.5  $\mu\text{g./ml.}$  or greater. A strain of *Brucella* isolated was inhibited by 1.56  $\mu\text{g./ml.}$  Preliminary results from studies on urinary excretion of kanamycin revealed a diminished excretion of the drug in patients with diminished renal function.

Immediate improvement in the clinical course, dramatic in some instances, was observed in those cases in which the causative organisms were sensitive in vitro to kanamycin and easily accessible to the action of the antibiotic in vivo. A partial

TABLE IV  
Comparative Sensitivity of Various

Organisms studied	Kana- mycin	Neo- mycin	Peni- cillin	Strepto- mycin
Staphylococci (91 strains)				
% sensitive	100	100	35	35
% resistant	0	0	65	65
Coli-aerogenes group (21 strains)				
% sensitive	100	100	0	47
% resistant	0	0	100	53
<i>Proteus-Pseudomonas</i> group (17 strains)				
% sensitive	83	83	5	47
% resistant	17	17	95	53

or transient effect was observed in patients if there were focal abscesses not sterilized by the antibiotic or in mixed infections where there was overgrowth of resistant organisms. The latter was commonly seen in chronic infections of the urinary tract or in mixed infections of the skin where an overgrowth of *Pseudomonas* usually developed. Persistent focal infections were prevented from spreading to other parts of the body but necessitated institution of drainage in 2 patients with severe staphylococcal sepsis and focal abscesses. Organisms isolated from such cases did not show any evidence of increased resistance to kanamycin.

Urinary tract infections caused by such organisms as *E. coli* and the *Aerobacter-Klebsiella* group and most strains of *Proteus* responded favorably to kanamycin therapy with sterilization of the urine in 24 to 48 hours.

A *Salmonella* carrier and a patient with endocarditis due to *Salmonella panama* were treated with oral and intramuscular forms of kanamycin with sterilization of the cultures and clinical improvement. Both of these patients had failed to respond to intensive therapy with other antibiotics.

Kanamycin therapy was considered a failure in 2 patients with severe staphylococcal infections of the vascular system. These patients responded subsequently to another antibiotic. These observations were made during the early part of the study when the maximal dose given was 1.5 Gm. daily. One patient with acute brucellosis treated intensively with kanamycin failed to respond clinically.

Deaths observed during kanamycin therapy were mostly due to the complicated types of diseases associated with the infection being treated, such as far-advanced degenerative diseases of the cardiovascular, renal, and respiratory systems in adults and with the institution of therapy late in the course of the illness. Other causes of failure of kanamycin therapy were: (1) Presence of underlying advanced noninfectious systemic diseases of the body; (2) therapy begun in terminal state; (3) infection due to kanamycin-resistant organisms; (4) failure to sterilize walled-off infections; and (5) overwhelming infections.

Routine laboratory studies performed included a urinalysis every other day, a blood urea nitrogen and complete blood counts twice a week, thymol turbidity and cephalin flocculation tests before and after treatment. Control and post-treatment audiograms and caloric tests were done when the patients' condition permitted.

No drug rashes or drug fever were observed during the course of treatment. The blood urea nitrogen was elevated at the beginning of therapy in 18 patients, none of whom showed an increase in the blood urea level during kanamycin

TABLE IV

*Organisms to Kanamycin and Other Antibiotics*

Tetra- cycline	Erythro- mycin	Chloram- penicol	Baci- tracin	Novo- biocin	Poly- myxin	Vanco- mycin	Risto- cetin
38	60	82	91	97	0	100	95
62	40	18	9	3	100	0	5
57	0	57	0	0	77	0	0
43	100	43	100	100	23	100	100
35	0	35	0	17	23	0	0
65	100	65	100	83	77	100	100

therapy. There was no evidence of toxic effect of kanamycin on the liver or bone marrow in the dosages used in this group of patients.

Only 2 patients showed definite hearing loss. One patient with a degenerative renal disease received 53 Gm. of kanamycin over a period of 30 days. The other patient received two courses of kanamycin, a total of 69 Gm. given over a period of six weeks. Subjective hearing loss was noted in one patient but not supported by audiometric studies due to inability to cooperate. Two other patients had subjective evidence of ototoxicity although in one there was no control audiogram and the hearing loss was not greater than would be expected in the patient's age group. In the other, only a small amount of kanamycin was administered in contrast to large amounts of another ototoxic drug.

The local tolerance to intramuscular injections of kanamycin seemed to be similar to that of streptomycin. There was no evidence of local irritation in the cases in which kanamycin was instilled in abscess cavities, pleural spaces, and peritoneal and ventricular cavities.

#### SUMMARY AND CONCLUSIONS

A review of the pharmacological and clinical studies of the new antibiotic kanamycin is presented. A close correlation has been noted in most instances between the in vitro studies on the bacterial strains isolated from the patients and the in vivo effect of the antibiotic. Results have been generally satisfactory in infections due to a wide variety of organisms except to various streptococci, pneumococci, many strains of *Pseudomonas*, and *Brucella*. Kanamycin has been particularly active against staphylococci, *E. coli*, and the *Aerobacter-Klebsiella* group. Some strains of *Proteus* and an occasional strain of *Pseudomonas* have also been eliminated by kanamycin therapy. The antibacterial activity of kanamycin resembles that of neomycin to which it exhibits complete cross resistance.

The usual intramuscular dose of kanamycin has been from 1 to 3 Gm. daily in adults and from 12.5 to 50 mg./Kg. body weight per day in infants given in divided doses at intervals of 6 to 12 hours. The size of the dose has been determined by the severity of the illness being treated. The drug is rapidly absorbed following intramuscular administration and about 80 per cent is excreted in 24 hours.

Kanamycin has been administered orally to reduce the fecal flora prior to bowel

surgery and to lower the blood ammonia level in patients with liver failure. However, there is poor absorption from the gastrointestinal tract so that this route of administration is not satisfactory for systemic infections. Local instillation of the drug in concentrations of 2.5 mg./ml. has been used to supplement parenteral therapy.

An occasional patient has developed hearing loss as a result of kanamycin therapy. Evidence of auditory nerve damage has been demonstrated early only by audiometric studies but following prolonged therapy clinical evidence of deafness has been observed in a few cases. The ototoxicity may be reversible if recognized early and the drug stopped, but if the drug is continued it may be irreversible. Human pharmacological studies under way suggest a decreased urinary excretion rate of kanamycin in patients with diminished renal function. Many patients have granular casts in their urine during kanamycin therapy but definite evidence of serious renal damage has not yet been clearly demonstrated clinically.

#### BIBLIOGRAPHY

1. UMEZAWA, H.; TAKEUCHI, T.; HIKIJI, T.; YAMAZAKI, S.; ABE, S., AND TOKYAMA, H.: Biological studies on kanamycin, *J. Antibiotics* 10:107, May, 1957.
2. UMEZAWA, H.; UEDA, M.; MAIDA, J.; YAGISHITA, K.; KONDO, S.; OKAMI, Y.; UTAHARA, R.; OSATO, Y.; NITTA, K., AND TAKEUCHI, T.: Production and isolation of a new antibiotic, kanamycin, *J. Antibiotics, Ser. A* 5:181, Sept., 1957.
3. TISCH, D. E.; HUFTALEN, J. B., AND DICKISON, H. L.: Pharmacological studies with kanamycin, *Ann. New York Acad. Sc.* 76:44, Sept. 30, 1958.
4. CRON, M. J.; FARDIG, O. B.; JOHNSON, D. L.; PALERMITI, F. M.; SCHMITZ, H., AND HOOPER, I. R.: The chemistry of kanamycin, *Ann. New York Acad. Sc.* 76:27, Sept. 30, 1958.
5. GOUREVITCH, A.; ROSSOMANO, V. Z.; PUGLISI, T. A.; TYNDA, J. M., AND LEIN, J.: Microbiological studies with kanamycin, *Ann. New York Acad. Sc.* 76:31, Sept. 30, 1958.
6. BUNN, P. A.; BALCH, A., AND KRAJNYAK, O.: Clinical experiences with kanamycin, *Ann. New York Acad. Sc.* 76:109, Sept. 30, 1958.
7. CRONK, G. A., AND NAUMANN, D. E.: Clinical and laboratory studies with kanamycin, *Ann. New York Acad. Sc.* 76:308, Sept. 30, 1958.
8. RUIZ SÁNCHEZ, F., AND RUIZ SÁNCHEZ, A.: Kanamycin in anthrax, typhoid, paratyphoid, and brucellosis, *Ann. New York Acad. Sc.* 76:235, Sept. 30, 1958.
9. ICHIKAWA, T.: Kanamycin treatment of urinary infections, *Ann. New York Acad. Sc.* 76:242, Sept. 30, 1958.
10. DUBE, A. H.: Kanamycin in the treatment of chronic urinary tract infection, *Ann. New York Acad. Sc.* 76:265, Sept. 30, 1958.
11. HIGH, R. H.; SARRIA, A., AND HUANG, N. N.: Kanamycin in the treatment of infections in infants and children, *Ann. New York Acad. Sc.* 76:289, Sept. 30, 1958.
12. FINEGOLD, S. M.; WINFIELD, M. E.; ARONSOHN, R. B.; HEWITT, W. L., AND GUZE, L. B.: Clinical experience with kanamycin, *Ann. New York Acad. Sc.* 76:319, Sept. 30, 1958.
13. YOW, M. D., AND WOMACK, G. K.: The use of kanamycin in a staphylococcal epidemic in infants and children, *Ann. New York Acad. Sc.* 76:363, Sept. 30, 1958.
14. YOW, E. M., AND MONZON, O. T.: Laboratory and clinical evaluation of kanamycin in resistant bacterial infections, *Ann. New York Acad. Sc.* 76:372, Sept. 30, 1958.
15. THURMAN, W. G., AND PLATOU, R. V.: Some experiences with kanamycin in the treatment of salmonella and shigella infections, *Ann. New York Acad. Sc.* 76:230, Sept. 30, 1958.
16. WRIGHT, K. W.; RENZETTI, A. D., JR.; LUNN, J., AND BUNN, P. A.: Observations on the use of kanamycin in patients in a tuberculosis hospital, *Ann. New York Acad. Sc.* 76:157, Sept. 30, 1958.
17. BOWEN, J. F.; JONES, J. M.; NASH, E. S.; RILEY, E. A.; SIMPSON, D. G., AND McCLEMENT, J. H.: Clinical experience with kanamycin in chronic pulmonary tuberculosis, *Ann. New York Acad. Sc.* 76:163, Sept. 30, 1958.
18. ANON.: The Committee of Chemotherapy for Tuberculosis. Clinical studies of kanamycin treatment of pulmonary tuberculosis, *Ann. New York Acad. Sc.* 76:166, Sept. 30, 1958.
19. COHN, I., JR.: Kanamycin for bowel sterilization, *Ann. New York Acad. Sc.* 76:212, Sept. 30, 1958.

20. FALON, W. W., AND FISHER, C. J.: The effect of kanamycin on blood ammonia levels in cirrhosis, *Ann. New York Acad. Sc.* 76:196, Sept. 30, 1958.
21. CHALMERS, T. C.; SEBESTYEN, K., AND TIMBERLAKE, W. H.: The effect of kanamycin on the mental and electroencephalographic abnormalities and tremor in cirrhosis and pulmonary emphysema, *Ann. New York Acad. Sc.* 76:188, Sept. 30, 1958.
22. PRIGOT, A.; SHIDLOVSKY, B. A., AND CAMPBELL, E. A.: Intraperitoneal use of kanamycin as an adjunct in the therapy of established peritonitis and peritoneal contamination, *Ann. New York Acad. Sc.* 76:204, Sept. 30, 1958.
23. WINFIELD, M.; CRISP, G. O.; MAXWELL, M. H., AND KLEEMAN, C. R.: Nephrotoxic effects of kanamycin: A preliminary report, *Ann. New York Acad. Sc.* 76:140, Sept. 30, 1958.
24. BERMAN, L. B., AND KATZ, S.: Kanamycin nephrotoxicity, *Ann. New York Acad. Sc.* 76:149, Sept. 30, 1958.

# A Comparative Study of the Antibacterial Spectra of Eleven Antibiotics, Using Three *In Vitro* Susceptibility Tests

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Antibiotic susceptibility tests are widely used in clinical laboratories as a means of determining the relative sensitivity of an organism to various therapeutic agents. The information obtained by these methods has been extremely useful in assisting the physician to choose the most effective antibiotic. Therefore, it is extremely important that the methods employed for susceptibility testing should give consistently reliable information.

The primary objective of this project was to determine the reliability of each of the following methods for routine bacterial susceptibility testing: (1) filter paper disc agar diffusion technique; (2) tube or broth dilution procedure; and (3) the cylinder plate method. In this manner, it was possible to reveal the frequency, extent, and direction of the variations obtained as the result of the different procedures, especially in regard to the performance of commercially prepared discs.

In addition, the study would serve to demonstrate the susceptibility patterns of different groups of bacteria to various antibiotics.

## MATERIALS AND METHODS

Three hundred strains of gram-negative and gram-positive organisms were isolated from bacteriological specimens obtained from patients at Henry Ford Hospital. Each organism was tested with as many as six concentrations of 11 chemotherapeutic agents (see tables I and II).

The three methods (disc, tube, cylinder plate) are clearly discussed in detail by Grove and Randall<sup>1</sup> as well as Jackson and Finland.<sup>2</sup> In order to obtain the best comparison and evaluation of the different techniques, the experiments were performed with standardized seed culture and freshly prepared dilutions of antibiotics (table II) or commercially prepared discs (table I). The organisms were defined as being sensitive or resistant according to the following scheme: sensitive, a marked zone of inhibition around the discs of both high and low series; moderately sensitive, minimal if any detectable zone of inhibition around the low series disc and a distinct zone of inhibition around the high series disc; and resistant, absence of a zone of inhibition around the discs of both concentrations.

## RESULTS

The susceptibility of 200 strains of staphylococci to seven antibiotics by the three methods are shown in table III. Inspection of the data shows only relatively small differences in the values. Since it is unlikely that concentrations indicated on the discs and in the dilution series would be exactly the same, one could hardly expect exact correlation. Minor discrepancies (one dilution differences) occasionally would have altered the reporting in lieu of changing the sensitivity pattern from one category to another. This is of practical importance with organisms on the borderline between moderately resistant and resistant, since the latter interpretation would exclude therapy. The *in vitro* assessment of the borderline resistant strains

TABLE I  
Concentrations of Antibiotic Discs

Antibiotic	Low series, $\mu\text{g.}$	High series, $\mu\text{g.}$
Penicillin	2	10
Dihydrostreptomycin	10	50
Tetracycline	5	30
Chloramphenicol	5	30
Erythromycin	2	15
Novobiocin	5	30
Oleandomycin	2	15
Neomycin	5	30
Polymyxin	5	30
Nitrofurazone	100	100
Nitrofurantoin	100	100

would be more accurate if they were tested by several methods. This is extremely important, if not mandatory, in severe infections such as a staphylococcal septicemia.

Table IV shows the results of the susceptibility tests with the gram-negative bacilli. The data show a greater variation in the sensitivity pattern due to the different methods than the results obtained using similar procedures against the staphylococci. The greatest discrepancy occurred with dihydrostreptomycin using the tube dilution procedure. According to Garrod<sup>4</sup> and others, the levels of resistance to streptomycin with in vitro susceptibility tests may vary with changes in the size of the inoculum. Branch et al<sup>5</sup> have shown that the size of the inoculum is an important factor in changing the minimum inhibitory concentration in the tube dilution method, and that it also has a slight effect in the agar well method, but has not been found to exert any demonstrable changes in the reading of the disc results. Furthermore, as already noted,<sup>2,3</sup> additional factors, such as media, period of incubation, and choice of size of inhibition zone, may account for some of the variation in the results shown in table IV.

The data in table IV show dihydrostreptomycin and nitrofurazone to be the most effective antibiotics in vitro against the gram-negative bacilli. The least effective of the five agents was tetracycline. Previous experience has shown that penicillin is ineffective against this group of organisms. Occasionally high concentrations of penicillin will inhibit members of the genus *Proteus*.

TABLE II  
Concentrations Used for Tube Dilution and Cylinder Plate Assays

Antibiotic	Amount of antibiotic ( $\mu\text{g./ml.}$ ), dilution no.					
	1	2	3	4	5	6
Penicillin	1	2	10	20	40	100
Dihydrostreptomycin	1	10	20	40	100	
Tetracycline	1	5	10	30	60	
Chloramphenicol	1	5	10	20	30	60
Erythromycin	2	5	30	60		
Novobiocin	1	5	20	30	60	
Oleandomycin	2	2	15	30	60	
Polymyxin	1	5	30	60		
Nitrofurazone	5	25	50	100	150	300
Nitrofurantoin	5	25	50	100	150	300

TABLE III  
*Comparative Tests with Three Methods on 200 Staphylococci*

Method	Penicillin	Tetra- cycline	Dihydro- streptomycin	Chloram- phenicol	Oleando- mycin	Erythro- mycin	Novo- biocin
Disc							
Per cent sensitive*	21.2	42.4	44.3	90.0	97.7	93.4	100
Per cent resistant†	78.8	57.6	55.7	10.0	2.3	6.6	0
Tube Dilution							
Per cent sensitive	18.2	42.4	44.3	88.6	97.7	90.4	100
Per cent resistant	81.8	57.6	55.7	11.4	2.3	9.6	0
Cylinder Plate							
Per cent sensitive	22.7	39.4	44.3	87.1	95.5	93.4	100
Per cent resistant	77.3	60.6	55.7	12.9	4.5	6.6	0

\* Organisms inhibited by the range of concentrations listed in table I for each antibiotic.

† Those species not inhibited by the antibiotic concentrations shown in table I.

### DISCUSSION

Certain conclusions are apparent after an examination of the foregoing data. As might be expected from the data of other investigators,<sup>5,6</sup> discrepancies do occur among the in vitro results obtained by the three different methods. None of the procedures lend themselves to accurate quantitation, although reliability and accuracy can be increased by using more than one method.

It is not meant to imply that tests for susceptibility of bacteria to antibiotics have little or no value. It is more proper to infer from our experiences and those of other investigators that the tests can be used as a general qualitative guide to distinguish susceptible from nonsusceptible organisms, if certain recommendations are carefully followed.<sup>6</sup>

The disc procedure was designed for simplicity and not for a high degree of accuracy. The disc procedure will give the maximum amount of information with minimum time spent. This method is adequate if two discs with different concentrations (see table I) are employed. Furthermore, it should be emphasized that the manufacturers of discs must adhere to potency and performance tests and the user should employ a standard technique, in order to obtain reliable and reproducible results. We have observed that, whenever these controls are adhered to, there is good agreement in the results of the susceptibility tests.

TABLE IV  
*Comparative Tests with Three Methods on 100 Gram-Negative Bacilli*

Method	Tetra- cycline	Dihydro- streptomycin	Chloram- phenicol	Nitro- furazone	Nitro- furantoin
Disc					
Per cent sensitive*	36.2	80.0	67.5	82.0	54.5
Per cent resistant†	63.8	20.0	32.5	18.0	45.5
Tube Dilution					
Per cent sensitive	42.5	62.3	60.0	75.0	50.0
Per cent resistant	57.5	37.7	40.0	25.0	50.0
Cylinder Plate					
Per cent sensitive	37.5	73.8	60.0	73.0	63.6
Per cent resistant	62.5	26.2	40.0	27.0	36.4

\* Organisms inhibited by the range of concentrations listed in table I for each antibiotic.

† Those species not inhibited by the antibiotic concentrations shown in table I.

A small percentage of major discrepancies with penicillin discs prepared by two manufacturers still occurs. Since this antibiotic is widely used, we are employing penicillin discs of both firms on all susceptibility tests requesting this antibiotic. As a result, we hope to decrease the possibility of any major discrepancy that would otherwise occur.

The tube dilution method gives an end point reading and demonstrates the minimum inhibitory concentration (MIC) of the antibiotic. While the information is reliable within the limits of error inherent in such a procedure, the method requires considerable material and is time consuming. Therefore, it is employed on a routine basis only in situations wherein quantitative results or synergistic studies with two or more drugs are of considerable clinical importance.

The cylinder plate assays compared favorably with the results of the other two methods. Although this procedure compared favorably with the other methods, it is too laborious for routine testing. The cylinder plate method is recommended as an additional tool for the control laboratory.

#### SUMMARY

The antibiotic susceptibility patterns of 300 gram-positive and gram-negative organisms were determined by three methods. Our experience in correlating the disc procedure with the two other methods has been described. After four years of experience to control in vitro susceptibility tests, we would advocate that adequate controls (i.e., potency and performance tests) be applied in the preparation and marketing of discs. There is a need to standardize the procedures within each laboratory, in order to assure reproducible and reliable results.

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#### BIBLIOGRAPHY

1. GROVE, D. C., AND RANDALL, W. A.: *Assay Methods of Antibiotics; a Laboratory Manual*. New York, Medical Encyclopedia, Inc., 1955.
2. JACKSON, G. G., AND FINLAND, M.: Comparison of methods for determining sensitivity of bacteria to antibiotics in vitro, *A.M.A. Arch. Int. Med.* 88:446-460, 1951.
3. GREENBERG, L.; FITZPATRICK, K. M., AND BRANCH, A.: The status of the antibiotic disc in Canada, *Canad. M.A.J.* 76:194-198, 1957.
4. GARROD, L. P.: The bactericidal action of streptomycin, *Brit. M. J.* 1:382-386, 1948.
5. BRANCH, A.; STARKEY, D. H.; RODGERS, K. C., AND POWER, E. E.: Experience with controlling antibiotic sensitivity tests in Department of Veterans Affairs hospital laboratories in Canada. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, pp. 1125-1132.
6. BRANCH, A.; STARKEY, D. H., AND POWER, E.: Control of antibiotic sensitivity discs. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 107-110.

# Correlation Between Results of the Tube Dilution Method for Determining Bacterial Sensitivity to Antibiotics and the Results of the Administration of These Antibiotics to Patients with Staphylococcic Bacteremia

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The selection of the proper antibiotic is a major problem for the clinician who makes the diagnosis of *Staphylococcus aureus* coagulase-positive bacteremia. In vitro antibiotic sensitivity tests have been criticized as being inadequate because of the different milieu in which the antibiotic must act in vivo.<sup>1,2</sup> Despite this criticism, many physicians are greatly influenced by the results of commercial disc antibiotic sensitivity tests, which are used in most bacteriology laboratories.<sup>3,4</sup> Previous reports have stressed the lack of standardization of the disc method in testing antibiotic sensitivity of bacterial strains,<sup>5,6</sup> and the variability in the results reported from different laboratories is remarkable.<sup>7,9</sup> "False resistance" in many tests has been related to the rates of diffusion and deterioration of the antibiotic<sup>10</sup> and has deprived patients of an effective and possibly curative antibiotic. On the other hand, the results of repeated tube dilution tests represent a better agreement<sup>7,11,12</sup> and less tendency to err toward showing increased sensitivity.<sup>7</sup>

The variability and complexity of clinical infections and the pharmacological factors in their treatment might make it difficult to use clinical data as convincing evidence of reliability of the sensitivity test. However, in the face of a fatal illness, as a resistant staphylococcic bacteremia, any evidence of sensitivity is usually sought for and the patient is given the benefit of a trial with the particular antibiotic. In the present study, the reliability of the tube dilution sensitivity test was investigated by correlating the result of the sensitivity in vitro by the tube dilution method with the effect of administration of the various antibiotics to patients with staphylococcic bacteremia.

## MATERIALS AND METHODS

Case records of 100 consecutive patients with *Staph. aureus* coagulase-positive bacteremia treated at Milwaukee County General Hospital between 1952 to 1957 were studied. Positive blood cultures for *Staph. aureus*, coagulase positive, had been obtained at least once on all the patients and more than once on most of them during their acute illness. Most of these patients had either been seen in consultation or treated by one of us (B. A. W.). In each instance in which an antibiotic was administered to a patient, one of three results was agreed on by the two investigators: (1) "no response" when the patient died under antibiotic treatment or the blood culture remained positive or the temperature did not drop after three days of administration of this antibiotic; (2) "response" when blood culture became negative and the patient was cured; (3) "undetermined response" when a careful examination of the case by the two investigators did not reveal enough evidence for classification as either response or no response.

After this decision was made, the report of the in vitro sensitivity of the *Staph. aureus* to the administered antibiotic was noted.

The tube dilution sensitivity method had been used in all the cases. A heavy bacterial inoculum (1:100 dilution of an 18 hour culture, which is approximately one million bacteria) was added to the test dilution of antibiotic. The mean inhibitory concentration (MIC) was determined by the absence of gross visual turbidity after incubation for 18 hours. The concentrations ranged from 100  $\mu\text{g./ml.}$  to 0.38  $\mu\text{g./ml.}$  Separate charts were then drawn for every antibiotic, plotting its MIC in  $\mu\text{g./ml.}$  obtained in vitro against the type of clinical response determined as outlined in every case in which this antibiotic was administered.

In addition, each instance in which a blood culture was positive when a particular antibiotic was being given was recorded and plotted against the MIC of the antibiotic to that particular strain.

## RESULTS

The study revealed a significant correlation between the in vitro sensitivity of *Staph. aureus*, coagulase positive, by the tube dilution method to the various antibiotics and the clinical response obtained when these antibiotics were administered. The details regarding each antibiotic will be discussed separately.

*Penicillin.* Eighty-one patients received penicillin at one time during the course of their illness; 57 had no clinical response, 18 responded, and in 6 cases the response was undetermined (table I).

In none of the patients who responded was the MIC of penicillin higher than 3  $\mu\text{g./ml.}$ , or in other words, none of the patients in whom the MIC of penicillin was 6  $\mu\text{g./ml.}$  and higher had a clinical response.

A "clinical level" of sensitivity to penicillin can therefore be considered at MIC of 6  $\mu\text{g./ml.}$ , above which no response is to be expected as no "false resistance" was noted. Of the 26 patients who were sensitive in vitro (MIC 3  $\mu\text{g./ml.}$  and less), 8 did not give the expected good clinical response, i.e., in those 8 cases there was no correlation. They were cases 35, 50, 52, 57, 61, 65, 96, 97; a summary of the clinical pictures is presented in table II.

It is evident from reviewing the clinical pictures that if the virulence of the organism and the altered host susceptibility are taken into account, rationalization for their "no response" can be reached.

In summary, then, if the MIC of penicillin by the tube dilution method in cases of *Staph. aureus* coagulase-positive bacteremia is 6  $\mu\text{g./ml.}$  and higher, no response should be expected on administration of the drug as no false resistance has been noted. On the other hand, a few patients with MIC of 3  $\mu\text{g./ml.}$  and less did not respond as expected and were discussed.

*Chloramphenicol.* Chloramphenicol was used in 53 patients of whom 4 had a "response," 45 "no response," and 4 were "undetermined." Three of the latter lived and 1 died. Of the 50 patients with MIC of 6  $\mu\text{g./ml.}$  and higher, only 3 responded; 2 of them had a MIC of 6 and 1 of 12. Thus a MIC of 6  $\mu\text{g./ml.}$  could be considered as the level of sensitivity of the organism to chloramphenicol above which little or no response is to be expected.

Two patients (96, 97) revealed positive sensitivities in vitro ( $<0.38$  and 3  $\mu\text{g./ml.}$ , respectively) but did not respond clinically (for summary of these 2 cases see table II).

In general, the clinical response in patients who received chloramphenicol was poor and supported the sensitivity studies (see table I).

*Erythromycin.* Erythromycin was given to 43 patients, and a definite "response"

TABLE I  
Correlation Between Sensitivity to Various Antibiotics and the Clinical Result

Inhibitory conc., $\mu\text{g./ml.}$	Penicillin		Chloramphenicol		Erythromycin		Streptomycin		Chlortetracycline		Oxytetracycline		Tetracycline		Vancomycin		Novobiocin		Neomycin	
	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse
<.38	8, 27 39, 46, 47,* 74, 100	50, 57, 96	96	19, 31, 35	19,	5, 21, 32, 35, 39*	57										52, 49, 48	52, 69	12	
.38	15, 36, 92		82			19, 23	21, 52						70		11, 97		39	80	6, 48, 49, 65	
.75	18, 68	61, 97		32, 38 39,* 42,* 74, 77, 82, 92	54, 96	8	26, 56	33	27, 68				64, 74					32, 58	71	
1.5	83, 91, 93			45, 56, 69, 91	40,† 49, 52, 65	30, 80†	28,† 50†						31	66			97	2		
3	5,* 21, 24, 38, 42	35, 52, 65, 84†	97	28, 58*	25, 79	36	14, 29	28†							49				26	
6	45*	40, 55	38, 42,* 91	6, 12, 40,† 44, 49, 70, 75, 77, 79	26, 97	18	16	41										39*		
12		14, 17, 26, 29, 37, 78	45	7, 35, 52, 54, 58, 61, 65, 72, 76, 78, 80, 86, 94, 95, 98		6	6, 7, 25, 87	60												

TABLE I (Continued)  
Correlation Between Sensitivity to Various Antibiotics and the Clinical Result

Inhibitory conc., μg./ml.	Penicillin		Chloramphenicol		Erythromycin		Streptomycin		Chlortetracycline		Oxytetracycline		Tetracycline		Vancomycin		Novobiocin		Neomycin	
	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse	Re- sponse	No re- sponse
25	23, 28,† 44, 54, 72		31,* 39° 66	25, 26, 66			24°		69											
50	11, 13, 51, 58, 70, 71, 99			28, 63, 83, 87, 88, 89	11, 66				1, 10, 12											
100	12, 19, 25, 56, 76, 80, 82, 85, 94			9, 11, 41, 48, 59, 60, 69, 71, 73, 80, 90	30, 48, 71, 73, 87	1, 17, 20, 34, 79		17					79							
>100	1, 2, 4, 6, 7, 9, 20, 30, 34,† 41, 43, 59, 60, 62, 67, 73, 75, 81, 87, 88, 89, 95		62°	10, 12, 41, 59, 63, 75, 80, 86		2, 7, 14, 25, 62, 87		1, 12	17, 88, 89				60							

\* Undetermined; patient lived.  
† Undetermined; patient died.

TABLE II  
Case Histories of 21 Patients

Case	Cases in which no clinical response was obtained while in vitro sensitivity was at MIC of 3 $\mu\text{g.}/\text{ml.}$ or less	Antibiotics administered and their MIC in $\mu\text{g.}/\text{ml.}$
35	7 years old, congenital cystic disease of the lung and <i>Staphylococcus</i> pneumonia and bacteremia	Penicillin, 3; streptomycin, 3
50	50 years old, diabetic with multiple systemic abscesses and papillary necrosis of kidneys and pericarditis	Penicillin, <0.38
52	29 years old, 50% burn and staphylococcal bacteremia, developed also <i>Pseudomonas</i> endocarditis and died	Penicillin, 3; erythromycin, 1.5; novobiocin, <0.38
57	70 years old, <i>Staphylococcus</i> meningitis secondary to otitis media and <i>Staphylococcus</i> septicemia	Penicillin, <0.38; streptomycin, 3; chlortetracycline, <0.38
61	64 years old, metastatic carcinoma of the gastrointestinal tract and terminal <i>Staphylococcus</i> septicemia	Penicillin, 0.75
65	42 years old, cirrhotic with hepatic decompensation, died of terminal <i>Staphylococcus</i> septicemia	Penicillin, 3; erythromycin, 1.5; neomycin, 0.38
96	76 years old, found stuporous at his home, developed <i>Staphylococcus</i> pneumonia and died, most probably had a cardiovascular accident	Penicillin, 0.38; chloramphenicol, 0.38; erythromycin, 75
97	69 years old, broken hip, developed wound infection and died in spite of an apparent response of fever	Penicillin, 0.75; chloramphenicol, 3; novobiocin, 1.5; vancomycin, 0.38
49	50 years old, diabetic with bacterial endocarditis, osteomyelitis of spine, and meningitis responded only to intravenous novobiocin and died unexpectedly 11 days later	Erythromycin, 1.5; novobiocin, <0.38; neomycin, 0.38; vancomycin, 3
54	84 years old, cellulitis, eczema, herpes zoster, and very septic course, had to be given cortisone, penicillin, erythromycin; died	Erythromycin, 0.75
30	29 years old, white woman, died of an acute bacterial endocarditis ( <i>Staph. aureus</i> ) within 3 days after admission	Streptomycin, 1.5
26	83 years old, came for elective transurethral resection, developed bacterial endocarditis and septicemia due to <i>Staphylococcus</i> and died	Chlortetracycline, 0.75; neomycin, 3
56	2 years old, 50% burns, developed <i>Staphylococcus</i> septicemia, did not respond to chlortetracycline but did respond to erythromycin and skin grafting	Chlortetracycline, 0.75
70	41 years old, pulmonary tuberculosis, entered for segmental resection, had a stormy post-operative course with bleeding and needed re-exploration, had <i>Staphylococcus</i> in pleural cavity and septicemia	Tetracycline, 0.38
6	59 years old, white man, fractured hip and post-operative wound infection, developed <i>Staphylococcus</i> septicemia and died in spite of chlortetracycline, chloramphenicol, penicillin, streptomycin, and finally neomycin	Neomycin, 0.38
11	46 years old, 30% burns, developed <i>Staphylococcus</i> septicemia and died in spite of extensive antibiotic therapy with penicillin, chloramphenicol, erythromycin, and vancomycin	Vancomycin, 0.38
12	68 years old, white man, developed an acute <i>Staphylococcus</i> bacterial endocarditis and septicemia after a transurethral resection and died 3 weeks later	Neomycin, <0.38

Table II Continued on Page 753

TABLE II (Continued)

Case Histories of 21 Patients

Case	Cases in which no clinical response was obtained while in vitro sensitivity was at MIC of 3 $\mu\text{g.}/\text{ml.}$ or less	Antibiotics administered and their MIC in $\mu\text{g.}/\text{ml.}$
48	68 years old, ruptured appendix, was operated on and subsequent course was septic with positive blood and cerebrospinal fluid cultures; at autopsy, he was found to have ruptured gall bladder also	Novobiocin, <0.38; neomycin, 0.38
71	86 years old, hospitalized with a permanent cystostomy tube for 8 months, developed septicemia with <i>Staphylococcus</i> sensitive only to neomycin and novobiocin and died after 24 hours of treatment with neomycin	Neomycin, 0.75
25	62 years old, advanced pulmonary tuberculosis, came in for a follow-up cystoscopy because of previous bladder resections in 1934 and 1953, developed fatal <i>Staphylococcus</i> septicemia with renal involvement	Erythromycin, 3
79	79 years old, diabetic, had a fractured hip and open reduction, developed <i>Staphylococcus</i> wound infection, osteomyelitis, and septicemia and died in spite of vigorous chemotherapy	Erythromycin, 3

was obtained in 14, "no response" in 24, and the rest were "undetermined." Eighteen strains had a MIC of 6  $\mu\text{g.}/\text{ml.}$  and higher, and none had a clinical response, which is a good correlation. On the other hand, of 25 strains with MIC of 3  $\mu\text{g.}/\text{ml.}$  and less, 7 did not respond clinically and did not correlate with the in vitro sensitivity (see table I).

For a summary of the clinical picture of those cases, refer to table II, cases 52, 65, 96, 49, 54, 25, 79, where it can be noted that they were extremely poor-risk patients and were sensitive to other antibiotics besides erythromycin and still no response was obtained.

Again with erythromycin, the MIC of 6  $\mu\text{g.}/\text{ml.}$  can be considered as a level above which no clinical response should be expected.

*Streptomycin.* Three patients of 22 who received streptomycin had a clinical response, and their corresponding MIC values were 6, 3, and 0.75  $\mu\text{g.}/\text{ml.}$ , respectively. Three other patients with low MIC levels (3  $\mu\text{g.}/\text{ml.}$  and less) did not respond as expected (see table II, cases 35, 57, 30), and 3 were undetermined.

All the other 13 patients had resistant sensitivity levels of 6  $\mu\text{g.}/\text{ml.}$  and more and correlated with the in vitro sensitivity by lack of clinical response (table I).

*Tetracyclines.* Forty-one patients were treated with one of the tetracyclines (either chlortetracycline, oxytetracycline, or tetracycline); 15 responded and their sensitivities in vitro revealed MIC values of 6  $\mu\text{g.}/\text{ml.}$  and less.

In the 17 cases where a MIC of the antibiotic was higher than 6  $\mu\text{g.}/\text{ml.}$ , no clinical response was obtained (table I). On the other hand, cases 26, 56, 70, and 57 did not respond clinically although they were sensitive in vitro with respective MIC levels of 0.75, 0.75, 0.38, and <0.38 (see table II for their clinical summary).

*Vancomycin, Novobiocin, Neomycin.* These antibiotics were used on 25 patients and 10 had a clinical response. It should be pointed out, however, that these antibiotics were used on the extremely ill patients and, especially in the case of neomycin, on nearly terminal patients after a trial with other antibiotics. In none

TABLE III  
The Number of Positive Blood Cultures at Different Sensitivity Levels  
of the Various Antibiotics

Inhibitory conc., $\mu\text{g.}/\text{ml.}$	Peni- cillin	Chloram- phenicol	Erythro- mycin	Chlortet- racycline	Oxytetra- cycline	Tetra- cycline	Strepto- mycin	Neo- mycin
<.38	1		1	1				
.38	1							1
.75				1				1
1.5			3				1	
3	3			1				1
6	1	3	1					
12	4	6	1	1	1		1	
25	3	4	1					
50	3	1	1					
100	5	6	4	1		1	2	
>100	11	3					1	

of them was the MIC more than 6  $\mu\text{g.}/\text{ml.}$ , i.e., all the strains were sensitive in vitro (table I). Ten patients did not respond; they were cases 52, 65, 11, 97, 49, 26, 6, 71, 48, and 12 described in table II.

Finally, all the positive blood cultures obtained during the administration of different antibiotics have been counted and plotted against the MIC of the antibiotic to that particular organism (table III). Positive blood cultures were obtained in 66 instances when the MIC was 6  $\mu\text{g.}/\text{ml.}$  and more and in 16 instances when the MIC was 3  $\mu\text{g.}/\text{ml.}$  and less.

#### DISCUSSION

The results obtained with every antibiotic separately can be compiled into one graphic record in which the different levels of sensitivity of all the strains to all the antibiotics used are plotted against the percentage of clinical responses obtained at every MIC separately.

Figure 1 clearly shows that a reliable level for evaluating whether or not a clinical response can be expected in vitro is a MIC of 6  $\mu\text{g.}/\text{ml.}$  At a level of 6

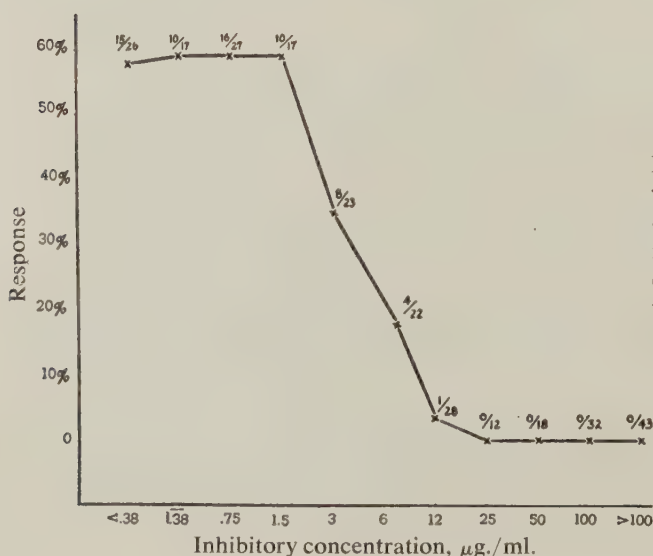


FIG. 1. Per cent of responses at various inhibitory concentrations of the antibiotics. The denominator in the fractions is the total number of observations and numerator is the number of responses at that particular minimum inhibitory concentration.

TABLE IV

*Response with MIC of 3  $\mu\text{g./ml.}$  or Less and 6  $\mu\text{g./ml.}$  or More*

	Total	Response	No response	Undetermined
Number of times MIC was 3 $\mu\text{g./ml.}$ or less	110	59	38	13
Number of times MIC was 6 $\mu\text{g./ml.}$ or more	155	5	140	10

$\mu\text{g./ml.}$  or more, there was a response in only 5 patients of 155; 4 of them had a MIC of 6  $\mu\text{g./ml.}$  (see table IV).

This is in contradiction to previous reports by Fisher and his associates<sup>24</sup> and Rogers<sup>13</sup> that many patients with penicillin-resistant *Staphylococcus* respond to penicillin. At the level of 3  $\mu\text{g./ml.}$  and less, 59 good responses correlated satisfactorily with the sensitivity studies. However, in 38 instances (21 patients, table II) antibiotics were used and no response was obtained, although the sensitivity study revealed MIC of 3  $\mu\text{g./ml.}$  and less.

This is in accordance with abundant evidence to indicate that antimicrobial therapy has not been successful in dealing with a number of serious *Staphylococcus* infections in hospitalized patients. The intracellular parasitism as a factor in drug resistance has been discussed previously.<sup>14, 15</sup> McDermott<sup>23</sup> believes in the presence of a serum protein constituent that may have a profound effect on the antimicrobial itself not present in vitro and may inactivate it. Dubos<sup>16</sup> postulated that the local biochemistry of the *Staphylococcus* lesion differs profoundly from the over-all internal milieu of the animal host and that the organisms may exist in a metabolic state in which they are relatively insusceptible to drug action at such local sites of infection.

Thus our therapeutic failures should not be ascribed to drug resistance alone but also to the basic underlying disease on which *Staphylococcus* is superimposed and to circumstances that lead to altered host resistance.

Perhaps the most striking point brought out by this study is that in practically no case was a therapeutic result obtained when the organism was resistant by the in vitro test, and the patient would not be denied an effective antibiotic because of false resistance, as was often the experience with the disc-type sensitivity tests.

#### SUMMARY AND CONCLUSION

1. One hundred consecutive case records of staphylococcic bacteremia seen at Milwaukee County Hospital were studied and the clinical responses to the administration of different antibiotics were determined.

2. The reports of the sensitivity of the *Staphylococcus* to the administered antibiotic as determined by the tube dilution method were examined. These yielded an objective correlation between the results of in vitro determinations done by the tube dilution method and the administration of antibiotics to patients.

3. In no case did a clinical response occur when the in vitro results showed the organism to be resistant to an antibiotic, but there was not always a clinical response when the organism was sensitive. This suggests that the clinician should not give patients antibiotics to which his infecting staphylococci are resistant in vitro by the tube dilution method. It further suggests that in vitro sensitivity by

this method is no guarantee of clinical results and that host factors and complications must always be considered.

## BIBLIOGRAPHY

1. BLISS, E. A., AND TODD, H. P.: A comparison of eight antibiotic agents in vitro, *J. Bact.* 58:61-72, 1949.
2. STONE, R. L.; BONIECE, W. S., AND CULBERTSON, C. G.: A comparison of the in vitro and in vivo effects of erythromycin, penicillin, and oxytetracycline against resistant staphylococci, *Antib. & Chemo.* 5:574-581, 1955.
3. GOLDIN, M., AND DAVIDSON, I.: Laboratory control of antibiotic therapy. A practical method for the general hospital, *Mod. Hosp.* 82:92, 1954.
4. TUNEVALL, G., AND ERICSSON, H.: Sensitivity tests by disc method as a guide for chemotherapy, *Antib. & Chemo.* 4:886-893, 1954.
5. BRANCH, A.; STARKEY, D. H.; RODGERS, K. C., AND POWER, E. E.: Experience with controlling antibiotic sensitivity tests in Department of Veteran Affairs hospital laboratories in Canada. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, pp. 1125-1132.
6. RODGERS, K. C.; BRANCH, A.; POWER, E. E.; STARKEY, D. H.; GREGORY, E.; MURRAY, R., AND HARROP, J.: Antibiotic therapy: correlation of clinical results with laboratory sensitivity tests, *Canad. M.A.J.* 74:605-612, 1956.
7. HOFFMAN, R. V.; JACKSON, G. G., AND TURNER, M. P.: Reliability of antibiotic sensitivity tests as determined by a survey study, *J. Lab. & Clin. Med.* 51:873-882, 1958.
8. LONG, P. H.: Bacterial resistance as a factor in antibiotic therapy, *Bull. New York Acad. Med.* 28:809-816, 1952.
9. BRANCH, A.; STARKEY, D. H., AND POWER, E. E.: A correlation of various antibiotic in vitro sensitivity tests. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, pp. 407-417.
10. BRAUDE, A. I., AND DOCKRILL, M.: Inaccuracies in disc method for determining bacterial sensitivity to Aureomycin, *J. Lab. & Clin. Med.* 40:149-155, 1952.
11. JACKSON, G. G., AND FINLAND, M.: Comparison of methods for determining sensitivity of bacteria to antibiotics in vitro, *A.M.A. Arch. Int. Med.* 88:446-460, 1951.
12. WAISBREN, B. A.; CARR, C., AND DUNNETTE, J.: The tube dilution method of determining bacterial sensitivity to antibiotics, *Am. J. Clin. Path.* 21:884-891, 1951.
13. ROGERS, E. E.: The current problem of staphylococcal infections, *Ann. Int. Med.* 45:748-781, 1956.
14. MACKANESS, G. B.: The action of drugs on intracellular tubercle bacilli, *J. Med. Research* 10:407, 1903-1904.
15. SHAFFER, J. M.; KUCERA, C. J., AND SPINK, W. W.: The protection of intracellular brucella against therapeutic agents and the bactericidal action of serum, *J. Exper. Med.* 97:77, 1953.
16. DUBOS, R. J.: *Biochemical Determinants of Microbial Diseases*, Cambridge, Mass., Harvard University Press, 1954.
17. BARBER, M., AND BURSTON, J.: Antibiotic resistant staphylococcal infection: a study of antibiotic sensitivity in relation to phage, *Lancet* 2:578, 1955.
18. FINLAND, M.; FRANK, P. F., AND WILCOX, C.: In vitro susceptibility of pathogenic staphylococci to 7 antibiotics (penicillin, streptomycin, bacitracin, polymyxin, aerosporin, Aureomycin, and Chloromycetin) with note on changing resistance of staphylococci to penicillin. *Am. J. Clin. Path.* 20:325, 1950.
19. FINLAND, M., AND HAIGHT, T. H.: Antibiotic resistance of pathogenic staphylococci: study of five hundred strains isolated at the Boston City Hospital from October, 1951 to February 1952, *A.M.A. Arch. Int. Med.* 91:143, 1953.
20. KLEIGER, B., AND BLAIR, J. E.: Correlation between clinical and experimental findings in cases showing invasion of the blood stream by staphylococci, *Surg., Gynec. & Obst.* 71:770, 1940.
21. FINLAND, M.: Present status of tests for sensitivity of bacteria to antimicrobial agents, *Am. J. Clin. Path.* 25:1333-1336, 1955.
22. WAISBREN, B. A., AND STRELITZER, C. L.: A five year study of the antibiotic sensitivities and cross resistances of staphylococci in a general hospital. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 350-364.
23. MCDERMOTT, W.: The Nature of the Lesion and the Response to Antimicrobial Therapy in Evaluation of Chemotherapeutic Agents, edited by C. M. McLeod, New York, Columbia University Press, 1949, p. 92.
24. FISHER, A. M.; WAGNER, N. H., JR., AND ROSS, R. S.: Staphylococcal endocarditis, *A.M.A. Arch. Int. Med.* 95:451, 1955.

# Pseudomembranous Enterocolitis, A Growing Menace

## An Autopsy Study

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The purpose of this paper is to present the evidence for our belief that pseudomembranous enterocolitis is an increasing menace. We hope this will facilitate the diagnosis of a disease that is notably deadly, most difficult to treat, and commonly diagnosed late in its course, if at all. Since successful therapy depends on prompt use of vigorous measures, it is considered essential that physicians be aware of any increase in the incidence of this disease—the sooner to make a correct diagnosis.

Information from the literature regarding the frequency of occurrence of pseudomembranous enterocolitis tends to be confusing. While some current articles<sup>1, 5, 7, 10</sup> suggest that the disease is more common, conclusions are often based chiefly on clinical observations. Since it is imitated by such varied diseases as acute pancreatitis, infectious diarrhea, peritonitis, coronary occlusion, and cerebral vascular accidents, and since there is presently no positive test for the disease during life (save passage of the membrane per rectum, a rare occurrence), we believe that conclusions drawn from such data could be misleading.

Reports<sup>2, 4, 6, 8</sup> based on autopsy studies of pseudomembranous enterocolitis, on the contrary, generally fail to show an increased frequency of occurrence. The most important of these studies is by Pettet,<sup>8</sup> who reviewed the autopsy cases at the Mayo Clinic from 1925 to 1952. She divided the study into an initial 13 year period, when 45 cases were found, and a second period from 1939 to 1952, with 49 cases. This latter group includes the period of the introduction and early use of antibiotic agents, stopping approximately at the time when broad-spectrum antibiotics were introduced. She believed that the use of antibiotics was not associated with an increase of pseudomembranous enterocolitis.

No one, to our knowledge, has since repeated Pettet's method of study with a review of autopsy protocols from one institution, starting prior to the introduction of the antimicrobial agents and bringing such a study up to date.

This factor of currency is, we feel, the key to the discrepancy, since the period from 1952 to 1953 is approximately the time when the use of broad-spectrum antibiotics became more widespread. It is also the period when antimicrobial-resistant staphylococci, believed by some to be important in the production of pseudomembranous enterocolitis, became more common as the cause of severe infections in hospitals.

### METHODS OF STUDY

Approximately 7000 autopsy protocols at the New England Deaconess Hospital Laboratory of Pathology, reported from 1928 to date, were studied for evidence of pseudomembranous enterocolitis. This was accomplished by reviewing the diagnoses as recorded and checking both the gross and microscopic descriptions of the gastrointestinal tract.

### ANALYSIS OF SOURCES

We are aware that by using autopsy material for study, we lose patients who

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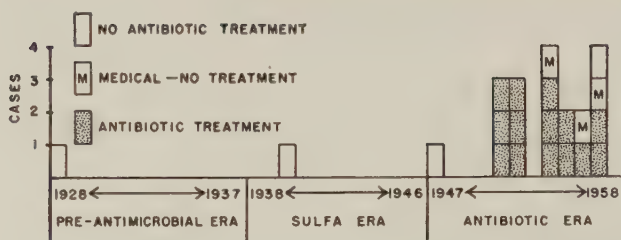


FIG. 1. The occurrence of 21 cases of pseudomembranous enterocolitis by years is shown. Eighteen are noted since 1951. Seven patients were not treated with antibiotics; three were medical.

survived through natural resistance or proper treatment. This probably results in selection of the more severe but, perhaps, more important cases. We recognize also that the hospitals served by this laboratory, namely, the New England Deaconess and the New England Baptist Hospitals, admit patients with more complicated and serious diseases than some similar sized community hospitals, in which emergency, obstetric, and pediatric cases comprise a significant portion of the total. This is true because, in addition to the highly trained general staffs, these hospitals serve the Lahey, Joslin, and Overholt Clinics, each bringing its quota of special and difficult cases.

The laboratory has been under the same director (Dr. Shields Warren) throughout the period of our study, providing a good continuity of standards of diagnosis and professional skills. The make-up of the attending staff has likewise been unusually stable, an important factor in such a study. While the bed capacity has increased in both institutions, it has not been at such a rate or time as to influence our findings.

The autopsy permission rate has ranged from 60 to 65 per cent for many years, suggesting the possibility of perhaps an additional 15 cases or more from those deaths when autopsy permission was not obtained.

## RESULTS

Twenty-one cases of pseudomembranous enterocolitis were discovered in this 30 year study. The first case, appearing in 1928, is of especial importance, since it proves the awareness of this disease by the professional staff in the first year of the study. The same is true of the second case found in 1940, occurring well before the current interest in the disease.

Following a scheme used previously,<sup>3</sup> we divided the study into a pre-antimicrobial era (1928 to 1938), a sulfonamide era (1938 to 1947), and an antibiotic era (1947 to date). While there is inevitable overlap in this grouping, it has value for purposes of classification.

Figure 1 shows the incidence of pseudomembranous enterocolitis by years and includes data as to whether the case was medical or surgical and whether antibiotics were used.

One case is found in the pre-antimicrobial era (1928), one in the sulfonamide era (1940), and the remaining 19 are clearly in the antibiotic period of the study—18 since 1951, a date near the time of introduction of the broad-spectrum antibiotics. Since 1951, at least 2 cases are found yearly, with the exception of 1953.

## CLINICAL COMMENT

While the primary purpose of this paper is to present evidence for an increase in the occurrence of pseudomembranous enterocolitis, brief comments on certain clinical aspects of the disease may be justified.

*Location.* As in other studies, the primary lesion for which surgery was performed was commonly located in the gastrointestinal tract (12 of 18 cases); in eight instances, carcinoma was present at the surgical site.

Perhaps by coincidence, in 6 of the surgical cases, the disease was found at the site of the procedure. This point, if significant, has not received much comment in the current literature. In two instances, the duodenum was the primary site. One was a patient with severe rheumatoid arthritis, being treated at the time with cortisone and requiring subtotal gastric resection for carcinoma. The second was a diabetic patient, in whom severe sunburn produced acidosis. The appearance of a pseudomembranous inflammation in these situations when duodenal ulcer is prone to form is intriguing, with regard to possible pathogenesis of pseudomembranous enterocolitis.

*Antibiotics.* Antibiotics were not given in 7 of the 21 cases; 3 of these were medical. Of the 14 patients who received antibiotics, they were given preoperatively in 7, postoperatively in 13, and during both periods in 5.

Penicillin and streptomycin, usually in combination, were the agents chiefly used in the 14 treated patients. Oxytetracycline was used in 4 cases, but in 3 of these the use of the drug was terminal, being given for the symptoms of pseudomembranous enterocolitis and therefore, we believe, not a provocative factor. Tetracycline and erythromycin were each used twice and chloramphenicol once, but these were also given for treatment of symptoms of the disease and likewise probably were not causative. In short, the broad-spectrum antibiotics did not appear to have been important as a direct cause in these cases, although their use within the hospital may well have established a bacteriological "climate" in which the disease might thrive.

*Cultures.* Cultures were obtained before or after death in 20 of the 21 cases. Staphylococci were found in only 8, the remaining cultures showing chiefly gram-negative organisms commonly found in the gastrointestinal tract. Whether or not the *Staphylococcus* was important in all or any of these 8 cases, we cannot say.

*Factors Possibly Tending to Produce Pseudomembranous Enterocolitis.* In addition to the presence of cancer (8 times), diabetes (6 times), and congestive heart failure (6 times), all factors tending to facilitate infection, two other influences previously noted<sup>3</sup> were found: (1) in 9 of the 18 patients who were operated on, the surgical procedure was performed in the presence of an established infection; and (2) 5 of 18 surgical patients had two major surgical procedures carried out within 16 days.

## DIAGNOSIS

Our findings re-emphasize the great difficulties in the diagnosis<sup>9</sup> of this condition during life. In 12 of the 21 cases, it was considered that there was little warning that pseudomembranous enterocolitis was imminent or even present. While shock was a prominent feature in 15 cases, it was terminal in 7, and in only 2 did it appear early.

Diarrhea was present in 16 of the 21 cases, but was essentially terminal in 6, while it was entirely absent in 5.

The temperature curve frequently failed to provide a satisfactory warning of the onset or to indicate the degree of severity of the disease. In 3 of the 21 cases, no fever was present at any time. In 10, it appeared only as a terminal sign—rising only to 101 F. in 3 and between 104 and 107 in 7.

A final point concerns the patients' mental state. Confusion, irritability, and even combativeness were prominent in 14 of the 21 patients. This was evident from the nurses' notes in those patients not seen by us, the changes appearing three to five days before any other sign of pseudomembranous enterocolitis was evident. Five of the 21 patients died within two days of admission to the hospital, which gave little opportunity for estimate of any early mental symptoms. Thus 14 of the 16 remaining patients presented confusion, irritability, or combativeness to a significant degree relatively early in the course of the disease. As a result, when such behavior in a postoperative patient is not explained by his native personality or by drugs, we now consider this to be an important warning of impending pseudomembranous enterocolitis.

#### SUMMARY

1. Approximately 7000 autopsy protocols from the Laboratory of Pathology, the New England Deaconess Hospital, covering the period from 1928 to 1958 have been reviewed.

2. From this group, 21 proved cases of pseudomembranous enterocolitis were noted—1 occurring in the pre-antimicrobial era, 1 in the sulfonamide era, 1 early in the antibiotic era, and the remaining 18 since 1951.

3. We believe that significant evidence of an increase in the occurrence of pseudomembranous enterocolitis in these autopsied cases is presented.

4. This increased incidence of the disease since 1951 is not explained by increased patient load, change in hospital techniques, nor change in responsible personnel.

5. This study does not directly implicate either the broad-spectrum antibiotics or staphylococci as significant causes of pseudomembranous enterocolitis.

6. Certain clinical features are mentioned; special emphasis is placed on the difficulty of diagnosing pseudomembranous enterocolitis and the possible value of mental confusion, irritability, or combativeness as important warning signs.

#### BIBLIOGRAPHY

1. COOPER, D. R.; FERGUSON, L. K., AND PESSER, J. F.: Acute enterocolitis, *Am. Surgeon* 23:165-177, Feb., 1957.
2. DEARING, W. H., AND HEILMAN, F. R.: Micrococcic (staphylococcic) enteritis as complication of antibiotic therapy; its response to erythromycin, *Proc. Staff Meet., Mayo Clin.* 28:121-134, March 11, 1953.
3. FOSTER, F. P., AND DADEY, J. L.: Management of postoperative diarrheas and infections, *Am. J. Surg.* 92:770-780, Nov., 1956.
4. HULTBORN, K. A.: Acute postoperative enterocolitis. Report of seven cases and review of literature, *Acta chir. Scandinav.* 111:29-44, 1956.
5. JOHNSTON, J. H., JR.; BRANNAN, C. D., AND HEARD, K. M.: Pseudomembranous enterocolitis, *Surgery* 39:975-981, June, 1956.
6. KLECKNER, M. S., JR.; BARGEN, J. A., AND BAGGENSTOSS, A. H.: Pseudomembranous enterocolitis: clinicopathologic study of 14 cases in which disease was not preceded by operation, *Gastroenterology* 21:212-222, June, 1952.
7. NEWMAN, C. R.: Pseudomembranous enterocolitis and antibiotics, *Ann. Int. Med.* 45:409-444, Sept., 1956.
8. PETTET, J. D.; BAGGENSTOSS, A. H.; DEARING, W., AND JUDD, E. S., JR.: Postoperative pseudomembranous enterocolitis, *Surg., Gynec. & Obst.* 98:546-552, May, 1954.
9. PROHASKA, J. V.; GOVOSTIS, M. C., AND TAUBENHAUS, M.: Postoperative pseudomembranous enterocolitis: successful treatment with corticotropin (ACTH), *J.A.M.A.* 154:320-323, Jan. 23, 1954.
10. WEISMANN, R. E., AND TWITCHELL, E. B.: Acute pseudomembranous enterocolitis in the postoperative patient, *Ann. Surg.* 144:32-38, July, 1956.

# Preoperative Sterilization of the Colon

## Comparison of Various Antibacterial Agents. IV

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The use of antibacterial agents for preoperative sterilization of the colon continues to be an important form of preparation for patients undergoing elective surgery of the colon. Thirty agents have now been tested by a standardized method of study in a single laboratory, so that ready comparisons are possible between various agents.<sup>1-5</sup>

### METHODS

Patients were selected from the surgical service, provided they were not suspected of having a lesion in the colon. During the period of study they received only the antibacterial agent under study.

The selected patients were placed on a low residue diet and given a laxative and daily enemas. After a control stool was obtained, they were given the antibiotic of choice for 72 hours. Stools were collected daily during therapy and for one to three days after therapy for qualitative and quantitative bacteriological analyses and for sensitivity studies. Stool or serum levels were measured for some drugs.

*Bacteriological Techniques.* Bacteriological techniques were identical with those reported previously.<sup>2-4</sup> A weighed quantity of stool was serially diluted from 10<sup>2</sup> through 10<sup>10</sup> dilutions, and each dilution was inoculated as follows: one plate of blood agar incubated aerobically; one plate of blood agar incubated anaerobically by the hydrogen method; and one plate of MacConkey agar, incubated aerobically.

At appropriate intervals, the plates were counted and organisms identified through Gram stain smears and subcultures.

Sensitivity studies were conducted with discs containing 5 and 50  $\mu$ g. of the drug(s) under study.

At scattered and infrequent intervals, species of *Bacillus*, *Proteus*, and *Pseudomonas* were found.

### CLORPACTIN WCS-90

While not strictly available for preoperative preparation of the colon, Clorpactin has been advocated as a bowel antiseptic.<sup>6,7</sup> Because of the paucity of reports dealing with Clorpactin, it was decided to evaluate the agent in experimental animals using the recommended technique.

Six healthy normal dogs were anesthetized and the abdomen opened through a midline incision. A clamp was placed on the colon just distal to the ileocecal junction. Another clamp was placed 40 cm. distal to the first clamp. Colon contents were obtained for culture through a small incision in the colon between the clamps. The colon was irrigated with 500 ml. of sterile saline solution, which was allowed to run out the rectum. As the irrigation was completed, some saline was removed from the colon for culture. Then the 40 cm. segment of colon was irrigated with a

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TABLE I  
*Clorpactin Intestinal Sterilization Study—Quantitative Bacterial Evaluation*

Bacterial group	Colon contents	Terminal saline washings	Terminal clorpactin washings	Mucosal scrapings
Streptococci	1 x 10 <sup>9</sup>	71 x 10 <sup>3</sup>	No growth	12 x 10 <sup>1</sup>
Coliform bacteria	9 x 10 <sup>7</sup>	76 x 10 <sup>3</sup>	No growth	14 x 10 <sup>2</sup>
Clostridia	8 x 10 <sup>7</sup>	50 x 10 <sup>1</sup>	No growth	14 x 10 <sup>1</sup>
Bacteroides	No growth	21 x 10 <sup>1</sup>	No growth	No growth

solution containing 2 Gm. of Clorpactin in 500 ml. of warm tap water. This solution was allowed to distend the colon between the clamps and then the distal clamp was removed so that the solution could be expelled. The clamp was then reapplied and this procedure repeated, until all 500 ml. had been utilized. At the end of the irrigation, culture of the terminal Clorpactin washings was obtained from the colon between the two clamps. In 5 dogs the colon was opened and examined, and the mucosa was scraped for culture.

Streptococci were present in all colon contents (table I) and in all saline washings, though the count was decreased in 3 animals. No streptococci were found in the terminal Clorpactin washings, though all mucosal scrapings revealed streptococci. Coliform bacteria were present in all 6 control specimens and were still present after the saline washings (table I). No coliform bacteria were obtained in the Clorpactin washings, though coliform organisms were obtained from four mucosal scrapings. Clostridia were present in 5 control specimens and, after saline washings, in 4 (table I). No clostridia were recovered from the terminal Clorpactin washings, but clostridia were obtained from four mucosal scrapings. Bacteroides were present in only two control specimens but were recovered in 4 animals from the saline washings (table I). No bacteroides were recovered after the Clorpactin washings nor on the mucosal scrapings.

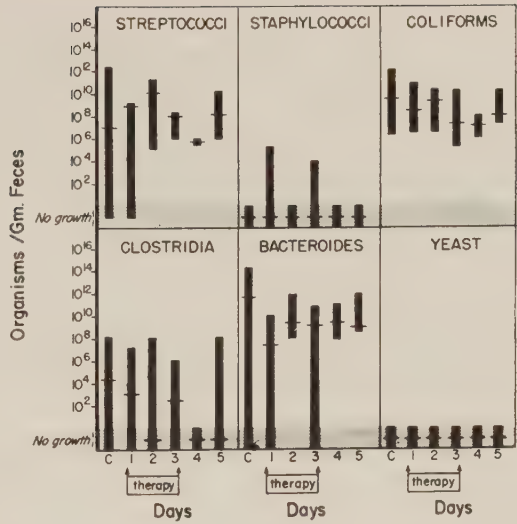
Neither staphylococci nor yeasts were recovered at any time.

*Discussion.* While the terminal Clorpactin washings did not show any streptococci, coliform bacteria, clostridia, or bacteroides, the mucosal scrapings revealed the persistence of almost all organisms. Thus, sterilization of the bowel was not achieved.

There are several objections to the use of this irrigation procedure in the operating room. If a patient can be prepared by the usual mechanical cleansing and preoperative antibacterial agents, there is no reason to bypass this means of intestinal antisepsis. If the patient is in critical condition or is subjected to an emergency operation, so that preoperative intestinal antisepsis cannot be carried out, then it is likely that the patient's condition will not tolerate an additional half hour of operating time required for irrigation with saline and Clorpactin. Since most emergency procedures are for obstructive lesions, the irrigations could not be discharged through the rectum and would have to be aspirated from the colon across the operative field. We have not studied the effect of Clorpactin in the peritoneal cavity, but we believe that the spillage of large quantities of such a solution that had been used to irrigate the colon would be highly undesirable. If the patient does not have an obstructive lesion, the resultant drainage of irrigating fluid onto the operating table or through a catheter into a pan would be undesirable. A false feeling of security resulting from dependence on the sterility of the terminal washings might tempt the surgeon to carry out procedures that would be detrimental to the patient.

Because of the technique required to utilize Clorpactin, we do not believe it should be used for intestinal antisepsis, except under unusual circumstances.

FIG. 1. Effect of furazolidone, given for three days, on bacteria in feces. Bars indicate maximum and minimum values for each day; cross marks indicate median value.



### FURAZOLIDONE

Furazolidone (Furoxone\*) was administered to 10 patients in dosages varying between 400 and 800 mg./day in tablet form. A final dosage schedule of 200 mg. in suspension four times a day for 72 hours was evaluated (fig. 1). This was felt to be the upper limit of safety. In the 6 patients who received this dosage, the usual studies were supplemented by studies of stool levels of furazolidone. No side effects were reported.

Streptococci were present in four control specimens and were subsequently found in the other 2 patients. There was no appreciable change in the count of streptococci during or after therapy. Staphylococci were not recovered from any control specimens but were found on the first day of therapy in 2 patients. They were again found at the end of therapy in 1 of these patients but were not otherwise recovered during the study. Coliform organisms were found in all control specimens and remained essentially unchanged. Clostridia were found in three control specimens and, on the first day of therapy, in 1 other patient. By the end of therapy, clostridia were present in 3 patients. Following therapy, clostridia were only found in the 2 patients who had them on the last day of therapy. Bacteroides were found in five control specimens and were subsequently found in the sixth patient. The growth of bacteroides was not significantly effected. No yeasts were recovered.

Streptococci were sensitive to 50  $\mu$ g. of furazolidone in the control and first day specimens from 2 patients and the first day specimen from 1 other patient (table II). All other streptococci recovered were resistant. Staphylococci recovered on one occasion were sensitive to 5  $\mu$ g. of furazolidone, but the staphylococci recovered on two occasions from the other patient were resistant. Coliform organisms in the control specimens were sensitive to 5  $\mu$ g. of the drug in 5 patients and to 50  $\mu$ g. in the sixth patient. All coliform organisms recovered after therapy were sensitive to 5  $\mu$ g. of furazolidone. All clostridia recovered were sensitive to 5  $\mu$ g. of furazolidone. Most bacteroides were sensitive to 5  $\mu$ g. of furazolidone, and this sensitivity was not significantly altered during therapy.

Stool studies were conducted so as to detect a minimum of 2  $\mu$ g. furazolidone/Gm.

\* The trade name of Eaton Laboratories for furazolidone is Furoxone.

TABLE II  
*Sensitivity to 5 and 50  $\mu$ g. of Furazolidone Before and After Therapy\**

Patient	Streptococci		Staphylococci		Coliform bacteria		Clostridia		Bacteroides	
	Before	After	Before	After	Before	After	Before	After	Before	After
Co	S <sup>50</sup>	R	NG	NG	S <sup>5</sup>	S <sup>5</sup>	S <sup>5</sup>	S <sup>5</sup>	NG	NG
De	R	R	NG	NG	S <sup>5</sup>	S <sup>5</sup>	NG	S <sup>5</sup>	S <sup>50</sup>	S <sup>5</sup>
Jh	NG	R	NG	NG	S <sup>5</sup>	S <sup>5</sup>	S <sup>5</sup>	NG	S <sup>5</sup>	S <sup>5</sup>
Jn	NG	R	NG	NG	S <sup>5</sup>	S <sup>5</sup>	NG	NG	S <sup>5</sup>	S <sup>5</sup>
Pa	S <sup>50</sup>	R	NG	NG	S <sup>5</sup>	S <sup>5</sup>	NG	NG	S <sup>5</sup>	S <sup>50</sup>
Rh	R	R	NG	NG	S <sup>50</sup>	S <sup>5</sup>	S <sup>5</sup>	NG	S <sup>5</sup>	S <sup>5</sup>

\* S,<sup>5,50</sup> sensitive to 5 or 50  $\mu$ g. of drug; R, resistant to drug activity; and NG, no growth.

stool. With the exception of one specimen received from each of 2 patients, no furazolidone was detected in the stool. Thus, the discrepancy between the apparent sensitivity of these organisms and their lack of control would be explained by the absence of drug from the colon.

*Discussion.* Because of the lack of control of the bacterial flora of the stool, furazolidone cannot be recommended for preoperative preparation of the colon.

#### RISTOCETIN

Ristocetin (Spontin\*) was administered orally to 5 patients, 250 mg. every hour for four hours, then 250 mg. every six hours for 72 hours. Nausea was noted in only 1 patient and was not severe enough to warrant discontinuing therapy.<sup>5,8</sup>

Streptococci, present in all control specimens, were removed from all but 1 patient at the end of therapy. Staphylococci were present in two control specimens but were not recovered subsequent to this. Coliform organisms were present in all patients and were not appreciably changed during the study. Clostridia were present in three control specimens but were not found at the end of therapy. Bacteroides were present in all control specimens and continued to be present in 3 patients. They appeared in 2 other patients on the day after therapy. Yeasts were not recovered.

All streptococci in the control specimens were sensitive to 5  $\mu$ g. of drug. Resistant forms appeared at intervals in 2 patients. Staphylococci were sensitive to 5  $\mu$ g. in 1 patient and 50  $\mu$ g. in the other patient. All coliform bacteria were resistant to ristocetin. Clostridia in the control specimens were sensitive to 5  $\mu$ g. in 2 patients and resistant in 1. Bacteroides recovered in the control specimens were sensitive to 5  $\mu$ g. in 2 patients, to 50  $\mu$ g. in 2 patients, and resistant in the fifth. By the end of therapy, all patients had resistant bacteroides.

*Discussion.* The failure to control coliform bacteria and bacteroides, the inadequate control of clostridia, and the slow response of the streptococci make ristocetin an unsatisfactory drug for preoperative preparation of the colon.

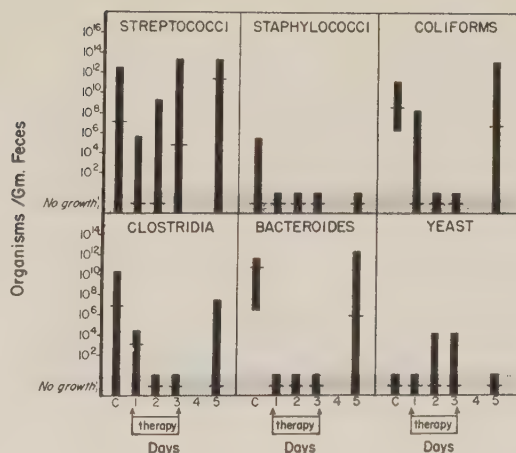
#### AMPHOTERICIN-NEOMYCIN

Amphotericin-neomycin was administered to 6 patients in the following dosage: amphotericin A and B (Fungizone<sup>†</sup>), 50 mg., in combination with neomycin, 1 Gm.,

\* The trade name of Abbott Laboratories for ristocetin is Spontin.

† The trade name of E. R. Squibb & Sons Division, Olin Mathieson Chemical Corp., for amphotericin is Fungizone.

FIG. 2. Effect of amphotericin-neomycin, given for three days, on bacteria in feces.



given every hour for four hours; then the combination given every six hours for a total of 72 hours (fig. 2). No disturbing side effects were reported.

Streptococci were present in four control specimens and were found on the first day of therapy in a fifth patient. By the end of therapy, streptococci were present in essentially undiminished concentration. Staphylococci were only found in one control specimen. Coliform organisms, found in all control specimens, had been removed from all but 1 patient on the first day of therapy. Clostridia were present in five control specimens and, on the first day of therapy, in the sixth patient. By the second day of therapy, clostridia were no longer recovered. Bacteroides were found in all control specimens but were not again found until two days after therapy. Yeasts were not present in any control specimens but were found on the second and third day of therapy in 1 patient and on the third day of therapy in another patient. They were not recovered after therapy was discontinued.

All streptococci in the control specimens were resistant to amphotericin. They were resistant to neomycin in 2 patients and sensitive at the 50  $\mu$ g. level in the other 2. Most streptococci found subsequent to this were resistant to both drugs. Staphylococci recovered in the one control specimen were resistant to amphotericin and sensitive to 5  $\mu$ g. of neomycin. Coliform organisms found in the control specimens were all resistant to amphotericin and all sensitive to 5  $\mu$ g. of neomycin. Organisms recovered after therapy showed essentially the same sensitivity. All clostridia were resistant to both drugs. All bacteroides in the control specimens were resistant to amphotericin and all but two were resistant to neomycin. Bacteroides recovered after therapy were resistant to both drugs. On the three occasions when yeasts were found, they were resistant to neomycin. Two specimens were sensitive to 5  $\mu$ g. of amphotericin and one to 50  $\mu$ g. of amphotericin.

*Discussion.* In view of the adequate and rapid control of coliform organisms, clostridia, and bacteroides and the absence of yeast or staphylococcal growth, the combination of amphotericin and neomycin can be considered one of the better drugs for preoperative preparation of the colon. The presence of an antifungal component makes this a particularly suitable drug for those conditions in which overgrowth of fungi might be feared.

#### KANAMYCIN

Kanamycin (Kantrex\*) was administered orally to 18 patients (1) in a dose of

\* The trade name of Bristol Laboratories Inc. for kanamycin is Kantrex.

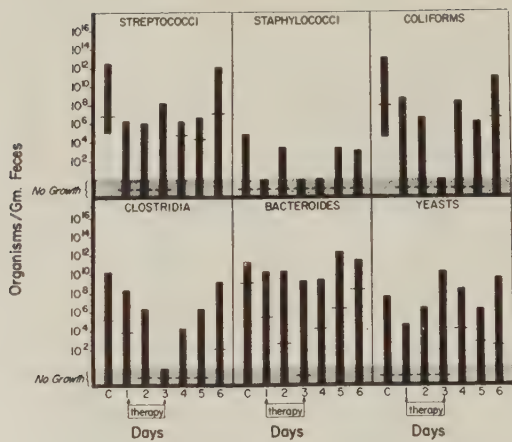


FIG. 3. The effect of kanamycin, given for three days, on the bacterial flora of the stool.

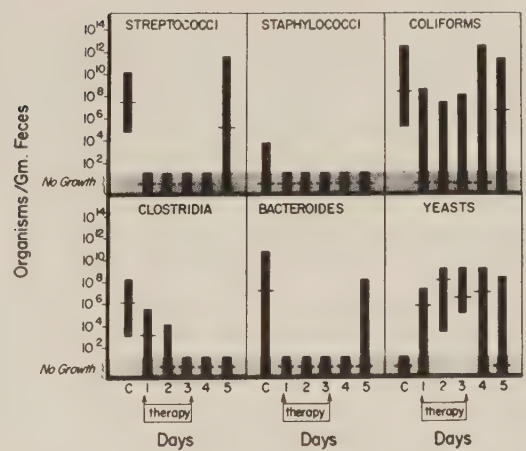
1 Gm. every hour for four hours and then 1 Gm. every six hours for 72 hours (fig. 3). Neither nausea, vomiting, diarrhea, nor other undesirable side reactions were noted.

Streptococci were present in all control specimens and had been removed from all but 3 patients by the end of therapy. Staphylococci were found in five control specimens but were not subsequently found in any of these patients. They appeared sporadically on three other occasions. Coliform bacteria were found in all control specimens but had been removed from 11 patients by the end of the first day of therapy and had been removed from all patients by the end of therapy. Clostridia were found in 12 control specimens and in an additional 3 patients during therapy. By the end of therapy, clostridia were absent from all but 4 patients. Bacteroides were found in 17 control specimens. Bacteroides were controlled in 11 patients but do not seem to have been appreciably effected in the remaining 6 patients. Yeasts were recovered in only one control specimen. By the end of therapy, yeasts had been found in 5 patients and appeared in 6 additional patients immediately after therapy.

Streptococci in the control specimens were sensitive to 5  $\mu$ g. of kanamycin in 5 patients, to 50  $\mu$ g. in 6 patients, and resistant in 7 patients. There were no significant changes in sensitivity in 7 patients, but there was increased resistance to kanamycin in 6 patients. The few staphylococci that were recovered were sensitive to 5  $\mu$ g. of drug. All coliform bacteria in the control specimens were sensitive to 5  $\mu$ g. of drug, and the majority of organisms continued to be sensitive at this level. Clostridia in the control specimens were sensitive in 8 patients and resistant in 4. Sensitive strains gave way to resistant strains in 5 patients. Except for four control specimens, all bacteroides were resistant to kanamycin. The 4 patients with sensitive strains subsequently had resistant strains.

*Discussion.* The control of streptococci, coliform bacteria, and clostridia, the infrequent occurrence of staphylococci, and the absence of significant overgrowth of yeasts all combine to make this a drug which can be highly recommended for preoperative preparation of the colon. The failure to control bacteroides, the major difference between this drug and neomycin, may be of minor importance, in view of the relative rarity of bacteroides as a cause of surgical infections. Since this is a single agent, which is not absorbed from the gastrointestinal tract and does not cause undesirable side reactions, it may be the most useful single agent available for preoperative preparation of the colon.

FIG. 4. The effect of ristocetin-neomycin, given for three days, on the fecal flora.



RISTOCETIN-NEOMYCIN

Ristocetin-neomycin was administered orally to 5 patients in the following doses: ristocetin, 200 mg., combined with neomycin, 500 mg., given every hour for four hours and then the combination given every six hours for a total of 72 hours (fig. 4). One patient had therapy discontinued because of diarrhea.<sup>5</sup>

Streptococci were present in all control specimens but were not subsequently recovered during therapy. Staphylococci were recovered in only one control specimen. Coliform organisms were found in all control specimens but were only found in 1 patient by the second day of therapy. Clostridia were present in all control specimens but were not recovered from any patients by the end of therapy. Bacteroides were present in four control specimens but were not again recovered during therapy. Yeasts were first present in all patients by the second day of therapy and continued to be present until one or two days after therapy.

All streptococci in the control specimens were sensitive to 5  $\mu$ g. of ristocetin. They were sensitive to 5  $\mu$ g. of neomycin in 3 and to 50  $\mu$ g. of neomycin in 2 patients. There was no change in sensitivity to ristocetin after therapy, but there was some increase in resistance to neomycin. The staphylococci found in only one control specimen were sensitive to 5  $\mu$ g. of either drug. All the coliform organisms were

TABLE III  
*Agents for Intestinal Antisepsis*

Unsatisfactory	Chloramphenicol Chlorquinaldol Chlorpactin WCS-90 Chlortetracycline Erythromycin Furazolidone	Novobiocin Oxytetracycline Penicillin V Ristocetin Sulfasuxidine	Sulfathalidine Tetracycline Tetracycline-nystatin Tetracycline V Thiostrepton
Satisfactory	Chlorquinaldol-neomycin	Neomycin	Oxytetracycline-neomycin
Recommended General Use	Amphotericin-neomycin Bacitracin-neomycin Kanamycin	Nystatin-neomycin Polymyxin B-neomycin	Sulfathalidine-neomycin Thiostrepton-neomycin
Special Use	Erythromycin-neomycin Novobiocin-neomycin	Ristocetin-neomycin	Tetracycline-neomycin

sensitive to 5  $\mu$ g. of neomycin, and all were resistant to ristocetin. All clostridia were sensitive to 5  $\mu$ g. of ristocetin, and all were resistant to neomycin. There was no change in this sensitivity during therapy. Bacteroides present in the four control specimens were all sensitive to 5  $\mu$ g. of ristocetin and resistant to neomycin in 3 patients. Bacteroides in the fourth patient were sensitive to 5  $\mu$ g. of neomycin. Sensitivity to ristocetin was unchanged after therapy, but resistance to neomycin had changed to sensitivity at the 5  $\mu$ g. level.

*Discussion.* The satisfactory control of the fecal flora with ristocetin-neomycin and the low incidence of gastrointestinal side reactions combine to make this a satisfactory combination for control of the bacterial flora of the feces. However, staphylococci resistant to other antibiotics are one of the prime indications for ristocetin, and if it were to be widely used for preoperative preparation of the colon, this chief usage might be lost. Thus, restriction of this drug to certain special cases would be its optimum indication.

#### DISCUSSION

A total of 30 intestinal antiseptics have been analyzed under standardized conditions in a single laboratory so that the relative efficacy of the various agents can be compared. The 30 drugs can be divided into three major classes (table III).

The agents considered unsatisfactory either did not provide adequate control of the bacterial flora of the feces, produced too many disturbing side reactions, or were subject to both of these criticisms. The satisfactory agents provided somewhat better control of the fecal flora and generally did not cause as many disturbing side reactions.

The recommended group is subdivided into those which are recommended for special use and those for general use. The control of the bacterial flora of the feces is roughly equivalent for all of these drugs, but there are other properties which make this subdivision desirable. The non-neomycin component of each of the combinations recommended for special use has certain properties that make it desirable to save them for cases in which specific bacteriological problems have developed or can be expected. The group recommended for general use are not absorbed from the gastrointestinal tract; generally are not widely used systemically and therefore are not likely to create antibiotic-resistant bacteria in the hospital population; provide rapid reduction of the bacterial flora; and do not produce side reactions. Neomycin is a component of all but one of the drugs in this category. The one remaining drug, kanamycin, is the only single agent classified as a preferred drug. Since kanamycin is a new agent, only time will determine whether its early promise as an intestinal antiseptic will make its use alone superior to that of neomycin in combination with some of the other drugs.

#### SUMMARY

Six antibacterial agents have been evaluated as agents for preoperative preparation of the colon by the same techniques as 24 previously reported.

Unsatisfactory agents for intestinal antisepsis are: chloramphenicol, chlorquinaldol, chlortetracycline, Clorpactin, erythromycin, furazolidone, novobiocin, oxytetracycline, penicillin V, ristocetin, sulfasuxidine, sulfathalidine, tetracycline, tetracycline V, teracycline-nystatin, and thiostrepton.

Satisfactory agents for preoperative preparation of the colon are: chlorquinaldol-neomycin, neomycin, and oxytetracycline-neomycin.

Recommended agents for special use for intestinal antiseptics are: erythromycin-neomycin, novobiocin-neomycin, ristocetin-neomycin, and tetracycline-neomycin.

Those agents that can be widely recommended for general use for preoperative preparation of the colon are: amphotericin-neomycin, bacitracin-neomycin, kanamycin, nystatin-neomycin, polymyxin B-neomycin, sulfathalidine-neomycin, and thiostrepton-neomycin.

#### ACKNOWLEDGMENTS

We would like to express our appreciation to Abbott Laboratories for ristocetin; Bristol Laboratories Inc. for kanamycin; Eaton Laboratories for furazolidone; Guardian Chemical Corporation for Clorpactin WCS-90; E. R. Squibb & Sons for amphotericin; and The Upjohn Company for neomycin.

Bacteriological determinations were conducted by Bette M. Beauclair, B.S., and Esther C. Alexander, B.S.

Patients studied were on the Louisiana State University Surgical Service at the Charity Hospital of Louisiana at New Orleans. Appreciation is expressed to the resident and nursing staff for their aid in this study.

#### BIBLIOGRAPHY

1. COHN, I., JR.: Kanamycin for bowel sterilization, *Ann. New York Acad. Sc.* 76:212, 1958.
2. COHN, I., JR., AND LONGACRE, A. B.: Preoperative sterilization of the colon. Comparison of various antibacterial agents. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, pp. 105-117.
3. COHN, I., JR., AND LONGACRE, A. B.: Preoperative sterilization of the colon. Comparison of various antibacterial agents. II. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, pp. 253-264.
4. COHN, I., JR., AND LONGACRE, A. B.: Preoperative sterilization of the colon. Comparison of various antibacterial agents. III. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 635-650.
5. COHN, I., JR., AND LONGACRE, A. B.: Ristocetin and ristocetin-neomycin for preoperative preparation of the colon, *A.M.A. Arch. Surg.* 77:224-229, Aug., 1958.
6. GLIEDMAN, M. L.; GRANT, R. N.; VESTAL, B. L., AND KARLSON, K. E.: Impromptu bowel cleansing and sterilization, *Surgery* 43:282-287, Feb., 1958.
7. GLIEDMAN, M. L.; GRANT, R. N.; VESTAL, B. L.; ROGERS, C. E., AND KARLSON, K. E.: Clorpactin, a surgical adjunct; antimicrobial and tumoricidal action. *In: Surgical Forum 1957*, Philadelphia, W. B. Saunders Co., 1958, pp. 104-108.
8. SHIDLOVSKY, B. A.; PRIGOT, A., AND TURELL, R.: The influence of ristocetin on the aerobic intestinal flora of man. A preliminary report. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 199-202.

# The Influence of Combinations of Ristocetin and Neomycin on Intestinal Aerobic Microflora

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The effect of the antibiotic ristocetin\* on aerobic microflora of the human intestinal tract was the subject of a previous report.<sup>1</sup> There was a clear-cut and significant reduction in fecal colonies of enterococci and staphylococci. Other investigators<sup>2-4</sup> have found this antibiotic clinically effective for serious infections due to gram-positive organisms. In a study of the potential of neomycin for intestinal antiseptis,<sup>5</sup> total bacterial colonies of the stool and the gram-negative counts were reduced, and this antibiotic produced also, although to a lesser degree, a decrease in the gram-positive counts.

This investigation extends the previous studies to determine the range of inhibition of two combinations of ristocetin and neomycin on the aerobic bacterial population and yeasts and fungi isolated from human feces.

## METHODS

We selected hospitalized patients who were free of any recent symptoms or history of gastrointestinal pathology. They received no medication that conceivably could interfere with the bacteriological assays for at least three days prior to or during the observation period.

Ristocetin was available as a powder in individual vials, each containing 250 mg. of the antibiotic. For oral administration, the powder was dissolved in water and the resultant solution divided for the prescribed dosage. Compressed tablets of 0.5 Gm. of neomycin† were employed.

The antibiotics were administered concurrently by the oral route, four times a day for one day, to two groups of 5 patients each. Table I details the regimen and total dosage. The first group of patients received a total of 400 mg. of ristocetin and 4 Gm. of neomycin (schedule A), while the second dosage (schedule B) resulted in a total of 4 Gm. of each of the antibiotics.

Fecal specimens not immediately processed were stored at -20 C. One Gm. of wet stool was serially diluted, and streak and pour plates were made from aliquots of appropriate dilutions. Cultures were developed on Tryptose 5 per cent blood agar, Endos agar, Azide agar with 5 per cent blood, *Staphylococcus* 110 medium, and Littmans-Oxgall agar.

The changes in colony numbers, isolated on various media from specimens taken 18 to 24 hours after termination of therapy, were compared with the bacteriological assays of pretreatment specimens. These changes are reported as percentages, for which the average is recorded in table II. Since assays disclosed large variations between counts in the control specimens of different subjects, the post-treatment results for each patient were compared with his individual control.

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This investigation was supported, in part, by a grant from the Abbott Laboratories, North Chicago, Ill., and the ristocetin was furnished by this company.

\* The trade name of Abbott Laboratories for ristocetin is Spontin.

† The trade name of The Upjohn Co. for neomycin is Mycifradin sulfate. The neomycin was supplied through the courtesy of this firm.

TABLE I

*Dosages of Ristocetin and Neomycin Employed for Intestinal Antisepsis*

Schedule	Number of patients	Medication		Total dosage, Gm.
A	5	Ristocetin—100 mg. plus neomycin—1 Gm.	4 times daily for 1 day	0.4 4.0
B	5	Ristocetin—1 Gm. plus neomycin—1 Gm.	4 times daily for 1 day	4.0 4.0

## RESULTS

The results of medication on the total bacterial population and different groups of intestinal microflora are presented graphically in figure 1.

Both schedules A and B produced consistently significant reduction (90 per cent or more) in the number of colonies of the total aerobic bacterial population, yielding average reductions of 95.5 and 96.1 per cent, respectively, 18 to 24 hours after the final dose of medication. Decreases in the gram-negative counts averaged 92.2 and 99.3 per cent, and in the gram-positive counts, 93.1 and 99.9 per cent. The larger dosage of ristocetin under schedule B, a total of 4 Gm., produced more consistent reductions than did the combination with the lower level of this antibiotic.

There appears to be a correlation between the increased quantity of ristocetin and the degree of reduction found in staphylococcal colony counts. Only the combination with the greater amount of ristocetin significantly decreased the staphylococcal numbers, with an average percentage of 99.9. The influence of the lower concentration of ristocetin on this group of organisms was reflected in inconsistent results (increases to 20 per cent and decreases to 97.7 per cent), yielding an average decrease of 50 per cent.

Neither dosage schedule demonstrated inhibition of yeasts and fungi. In 3 patients in each group, post-treatment specimens revealed no change as compared with the controls, and in 2 patients under each dosage there was evidence of increase in these microflora.

There were no allergic or other untoward reactions among the patients who received medication under this study.

TABLE II

*Effect of Ristocetin and Neomycin on Intestinal Microflora, Per Cent Reduction in 18 to 24 Hours Post-treatment Compared with Pretreatment Specimens*

Pa- tients	Total bacterial count		Gram- negative count		Gram- positive count		Staphylo- coccal count		Yeasts and fungi	
	Schedule A	Schedule B	Schedule A	Schedule B	Schedule A	Schedule B	Schedule A	Schedule B	Schedule A	Schedule B
1	-92.7	-93.1	-90.8	-99.9	-99.9	-99.9	-97.7	-99.9	N.C.	+99.9
2	-95.6	-96.7	-89.7	-96.7	-98.6	-99.9	-99.9	-99.9	N.C.	+16.7
3	-99.0	-99.9	-94.1	-99.9	-99.9	-99.9	+20.0	-99.9	+99.9	N.C.
4	-96.1	-90.8	-95.0	-99.9	-78.9	-99.9	-99.9	-99.9	N.C.	N.C.
5	-94.3	-99.9	-91.6	-99.9	-88.0	-99.9	7.3	-99.9	-99.9	N.C.
Av.	-94.3	-96.1	-92.2	-99.3	-93.1	-99.9	-57.0	-99.9	—	—

N.C. = no change.

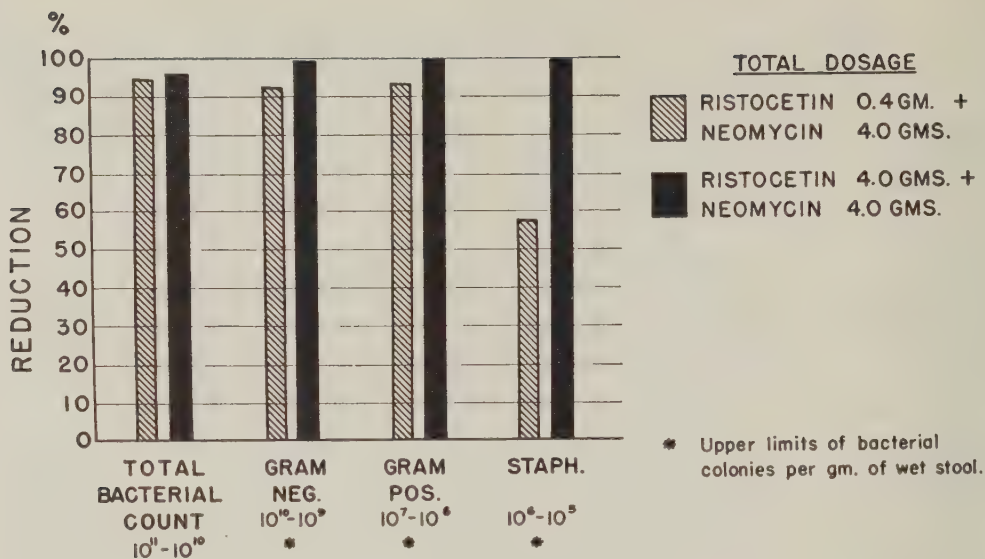


FIG. 1. Effect of ristocetin with neomycin on intestinal bacteria.

#### DISCUSSION

Both enterococci and staphylococci are in the category of aerobic gram-positive bacteria against which ristocetin has demonstrated pronounced inhibitory action. Neomycin is effective against most of the aerobic gram-negative species found in human stool. On the premise that each antibiotic might exert its respective influence on susceptible organisms, ristocetin in two dosage levels (100 mg. and 1 Gm.) was combined with 1 Gm. of neomycin and administered four times in one day. These antibiotics afford the additional advantage of relatively low absorption from the intestinal tract. They should thus exert a maximal effect on intestinal microflora provided they are not inactivated by the substrate.

From the highly consistent reduction of the aerobic bacterial population 18 to 24 hours post-treatment, it appears that a correlation exists between the variation in the dosage of the antibiotics and the level of decrease.

A reproducible, consistent reduction of 90 per cent or better in a period of 48 hours from initiation of medication is essentially what we are seeking and is considered a significant index to bacterial inhibition. This maximal reduction of fecal flora is sought as an aid in the cleansing of the intestinal tract prior to surgery.

#### SUMMARY

Two different quantities of ristocetin (400 mg. and 4 Gm.), each added to 4 Gm. of neomycin and administered in divided doses, were studied to determine the effect of the antibiotic combinations on the aerobic microflora of the human intestinal tract.

The combination of 4 Gm. of each antibiotic reduced the staphylococci by 99.9 per cent.

Assays of the total aerobic bacterial population and the gram-negative and gram-positive colonies, 18 to 24 hours after completion of treatment, revealed similar levels of reduction from both dosage levels of ristocetin when combined with 4 Gm. of neomycin.

No decrease in the number of colonies of yeasts and fungi was demonstrated by the combinations of antibiotics employed in this study.

No toxic manifestations were evident in any of the 10 subjects who received medication.

#### CONCLUSION

Combined ristocetin and neomycin, at a total dosage of 4 Gm. of each antibiotic in a 24 hour period, has a marked and consistent inhibitory effect on the intestinal microflora of man, including the staphylococci in its range of activity. This combination is ideally suited for presurgical preparation of the large bowel, where its suppression of staphylococci represents a distinct advantage.

#### BIBLIOGRAPHY

1. SHIDLOVSKY, B. A.; PRIGOT, A., AND TURELL, R.: The influence of ristocetin on the aerobic intestinal flora of man: a preliminary report. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 199-202.
2. TAYLOR, D. E.; SCHUMACHER, L. R., AND CALVY, G. L.: Ristocetin in the treatment of antibiotic-resistant staphylococcal pneumonia. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 180-186.
3. ROMANSKY, M. J., AND HOLMES, J. R.: Successful short-term therapy of enterococcal and staphylococcal endocarditis with ristocetin—seven patients: preliminary report. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 187-198.
4. BILLOW, B. W.; MARTORELLA, F. J.; LUPINI, B., AND PALEY, S. S.: Clinical observations on ristocetin: a preliminary report on its efficacy and toxicity in 20 unselected severe respiratory infections. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 447.
5. PRIGOT, A.; SHIDLOVSKY, B. A.; TURELL, R., AND MARMELL, M.: Presurgical preparation of the large bowel with neomycin and neomycin in combination with other antibiotics. I. Neomycin administered for various intervals and in combination with erythromycin. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, pp. 451-456.

# Kanamycin Used Alone and in Combination with Erythromycin for Intestinal Antisepsis

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Kanamycin, a water-soluble antibiotic,<sup>1</sup> was isolated from *Streptomyces kanamyceticus* by Umezawa of the University of Tokyo. The antibiotic is composed of two fractions, kanamycin A and B.<sup>2</sup>

Numerous investigators<sup>3-9</sup> have documented its in vitro and in vivo activity against species of *Salmonella*, *Shigella*, staphylococci, gonococci, and some strains of *Proteus* and *Pseudomonas*. It is relatively ineffective against streptococci.

Organisms resistant to penicillin, streptomycin, erythromycin, tetracycline, chloramphenicol, oleandomycin, and novobiocin have demonstrated susceptibility to kanamycin. The inhibitory range of kanamycin is similar to that of neomycin, and there appears to be a cross-resistance between the two antibiotics.<sup>1</sup>

The wide antibacterial spectrum of kanamycin and its low absorption from the gastrointestinal tract after oral administration indicated its possible use as an agent for antisepsis of the large bowel.

Erythromycin is primarily effective against gram-positive organisms, including the enterococci and staphylococci found in the large intestine. With a view to securing maximum reduction of the bacterial population of the gastrointestinal tract, the two antibiotics, kanamycin and erythromycin, were used as combined medication for a part of this study.

## METHODS

Kanamycin alone was administered in two different dosage schedules and was combined with erythromycin in the third, as shown in table I.

The assay of fecal specimens for intestinal microflora was based on a serial dilution of 1 Gm. of wet stool in sterile 0.1 per cent peptone. Samples of specific stool dilutions were used to make streak and pour plates before, during, and after medication.

Differential media were employed for colony counts (Tryptose blood agar base with 5 per cent blood, Endos medium, Azide agar with 5 per cent blood, *Staphylococcus* 110 medium, and Littmans-Oxgall agar). Colony counts of the organisms identified on differential media were recorded.

Hospitalized patients were screened, and those with any history or recent symptoms of gastrointestinal pathology were rejected. Those selected for this study had received no medication for at least three days prior to treatment that might interfere with the interpretation of results. No cathartics were used to obtain specimens, and all patients received the routine hospital diet.

## RESULTS

Percentage changes recorded were obtained by subtracting the number of colonies

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This investigation was supported, in part, by a grant from Bristol Laboratories Inc., Syracuse, N. Y., and this company furnished the kanamycin as a peroral preparation.

TABLE I

*Dosages of Kanamycin and Erythromycin Employed for Intestinal Antisepsis*

Schedule	Number of patients	Medication		Total dosage, Gm.
A	5	Kanamycin	0.5 Gm. 4 times daily for 1 day	2.0
B	5	Kanamycin	1.0 Gm. 4 times daily for 1 day	4.0
C	5	Kanamycin	1 Gm. }	4.0
		plus erythromycin	1 Gm. } 4 times daily for 1 day {	4.0

of microflora identified from specimens taken 18 to 24 hours after the final dose of medication from the number of colonies found in pretreatment stools. Table II shows the distribution of percentage changes, effected by two dosage schedules of kanamycin and the antibiotic combination, in the total bacterial population and different groups of microflora isolated. The average percentage changes are presented graphically in figure 1.

*Total Aerobic Bacterial Counts.* The greater quantity of kanamycin (4 Gm.) given alone produced consistent significant reductions, ranging from 85 to 97.1 per cent, with an average of 92.4 per cent, in the number of total aerobic bacterial colonies. The lower dosage of this antibiotic (2 Gm.) produced more variable reductions, 4.9 to 99.9 per cent, averaging 80.6 per cent.

Under the combination, 4 Gm. of kanamycin given simultaneously with 2 Gm. of erythromycin, decreases in the total aerobic bacterial population were more significant and consistent, 98.7 to 99.9 per cent, average 99.5.

*Gram-Negative Counts.* Comparison of the inhibitory influence of two dosage levels of kanamycin alone (2 Gm. and 4 Gm.) indicates that with the greater quantity of the antibiotic there was a correspondingly greater reduction in gram-negative colonies in post-treatment specimens. Under the 2 Gm. dosage, reductions in these bacteria ranged between 0 and 99.9 per cent, with an average change of 75.9 per cent. Four Gm. of kanamycin resulted in decreases ranging from 90.3 to 99.9, averaging 97.5 per cent.

Peroral dosage of 4 Gm. of kanamycin combined with 2 Gm. of erythromycin decreased the gram-negative colonies between 99.4 and 99.9 per cent, with an average reduction of 99.8.

*Gram-Positive Counts.* In 4 of the 5 patients receiving kanamycin at the 2 Gm. level, reductions in colonies of gram-positive organisms ranged from 66.7 to 99.9 per cent. However, the stool specimen of 1 patient, secured 18 to 24 hours post-treatment, revealed an increase of 99.9 per cent. The average percentage reduction for the group of 5 patients was therefore 44.4. The larger dose of kanamycin (4 Gm.) produced reductions in all 5 patients ranging from 52.3 to 99.6 per cent (average 82.2 per cent).

Fecal specimens from patients receiving 4 Gm. of kanamycin simultaneously with 2 Gm. of erythromycin exhibited more consistent and significant decreases in gram-positive colonies, which were estimated to range between 91.3 and 99.8 per cent, yielding an average reduction of 96.9 per cent.

*Staphylococcal Counts.* The colony counts of staphylococci followed the same pattern as was disclosed in the groups of organisms previously discussed. As the quantity of kanamycin was increased from 2 to 4 Gm., the suppression of the number of colonies was enhanced. The lower dosage schedule produced reductions rang-

TABLE II

Percentage Reduction in Various Fecal Microflora 18 to

	Total bacterial count			Gram-negative count		
	A	B	C	A	B	C
	— 4.9	—85.0	—98.7	—82.2	—99.9	—99.9
	—98.2	—91.9	—99.4	—98.4	—99.9	—99.9
	—99.9	—95.0	—99.9	0	—90.9	—99.9
	—99.9	—97.1	—99.9	—98.9	—97.3	—99.4
	—99.9	—91.7	—99.8	—99.9	—99.9	—99.7
Average per cent reduction	—80.6	—92.4	—99.5	—75.9	—97.5	—99.8

ing from 44.5 to 99.9 per cent, with an average of 88.7, while 4 Gm. of kanamycin resulted in decreases between 90.2 and 99.9 per cent, averaging 97.2 per cent.

The combination of kanamycin (4 Gm.) with erythromycin (2 Gm.) reduced the staphylococcal colony counts in post-treatment feces with assays ranging from 94.5 to 99.9 per cent, averaging a percentage change of 93.

*Yeasts and Fungi.* Variable results on yeasts and fungi were demonstrated by peroral kanamycin at both dosage levels. The post-treatment stool of 1 patient on the lower dosage revealed no change in these microflora. In 3 patients, yeasts and fungi increased by 35.7 to 99.9 per cent, and in the fifth subject, there was a decrease of 93.9 per cent. The higher dosage level of kanamycin produced decreases in the colony counts of all 5 patients, from 37.5 to 99.9 per cent, with an average reduction of 72.6 per cent.

Results of the combination of the two antibiotics on intestinal yeasts and fungi were too inconsistent to indicate any definite trend.

#### DISCUSSION

In the first few months after kanamycin was introduced into this country, extensive explorations of its properties were undertaken. Details of these initial investigations were presented at a symposium sponsored by the New York Academy of Science. From in vitro and in vivo experiments, kanamycin was found to inhibit the growth of staphylococci and to be effective to a limited degree against streptococci. It was also credited with the control of coliform organisms and clostridia in the stool.<sup>10</sup> Human infections due to *Salmonella* and *Shigella*<sup>8</sup> have responded to kanamycin, but it appears to be less active against species of *Pseudomona aeruginosa* in urinary tract infections.<sup>11,12</sup>

In contrast to the inhibitory range of kanamycin, erythromycin is capable of suppressing the gram-positive bacteria, which, in human stool, are principally enterococci and staphylococci.<sup>13,14</sup>

These findings led to the postulate that by combining kanamycin and erythromycin, each antibiotic would exert its influence on the respective susceptible organisms found in the human intestinal tract. The combination would therefore produce a greater reduction in the aerobic microflora of the stool than either agent alone.

The results of this study substantiate our hypothesis. Kanamycin with erythromycin not only effectively decreased the total aerobic bacterial count, but also those of the gram-negative, gram-positive (mostly enterococci), and staphylococcal colo-

TABLE II

24 Hours Post-treatment (Drug Schedules A, B, and C)

Gram-positive count			Staphylococcal count			Yeasts and fungi count		
A	B	C	A	B	C	A	B	C
+99.9	-52.3	-99.3	-44.5	-99.9	-74.5	0	-58.0	0
-99.9	-64.8	-94.3	-99.9	-90.2	-98.9	-93.9	-37.5	0
-83.2	-99.5	-99.9	-99.9	-99.9	-99.9	+99.9	-67.7	+90.0
-73.2	-94.5	-91.3	-99.2	-95.9	-94.1	+35.7	-99.9	-64.1
-66.7	-99.6	-99.8	-99.9	-99.9	-97.8	+99.9	-99.9	-93.3
-44.6	-82.2	-96.9	-88.7	-97.2	-93.0	+29.3	-72.6	-13.5

nies in fecal specimens taken 18 to 24 hours after the final medication. Results obtained with yeasts and fungi were too variable for definite conclusions.

During the period of this investigation, none of the subjects had any evidence of toxic or side reactions.

## SUMMARY AND CONCLUSION

Kanamycin, a broad-spectrum antibiotic with limited absorption on oral administration, was screened as a potential intestinal antiseptic.

The antibiotic alone was employed in two dosage schedules, 2 and 4 Gm. in four divided doses. The higher dosage level was combined with 2 Gm. of erythromycin in four divided doses for one day.

The higher dosage level of kanamycin reduced the total aerobic bacterial population and the gram-negative and staphylococcal counts. Its influence on gram-positive bacteria, including the enterococci, was less pronounced, and on yeasts and fungi was highly variable.

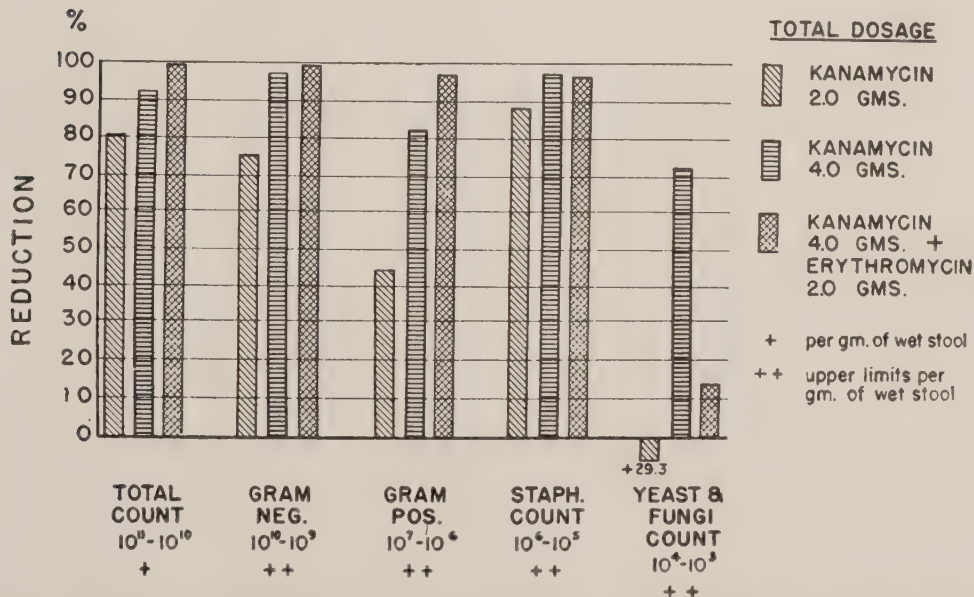


FIG. 1. Effect of kanamycin alone and in combination with erythromycin on the aerobic microflora of the stool.

The combination of kanamycin and erythromycin produced a consistent and significant decrease in colonies of the gram-positive group, as well as in the total aerobic bacterial count and the gram-negative and staphylococcal counts.

Kanamycin in combination with erythromycin manifested consistent reduction in the bacterial population of the gastrointestinal tract, indicative of its effectiveness as an intestinal antiseptic.

#### BIBLIOGRAPHY

1. GOUREVITCH, A.; HUNT, G. A., AND LEIN, J.: Antibiotic activity of kanamycin, *Antib. & Chemo.* 8:149, 1958.
2. CRON, M. J.; FARDIG, O. B.; JOHNSON, D. L.; PALERMITI, F. M.; SCHMITZ, H., AND HOOPER, I. R.: The chemistry of kanamycin, *Ann. New York Acad. Sc.* 76:27-30, 1958.
3. MARMELL, M., AND PRIGOT, A.: Observations on the treatment of acute gonococcal urethritis in the male with intramuscular kanamycin. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 804.
4. GOUREVITCH, A.; ROSSOMANO, V. Z.; PUGLISI, T. A.; TYNDA, J. M., AND LEIN, J.: Microbiological studies with kanamycin, *Ann. New York Acad. Sc.* 76:31-41, 1958.
5. HUNT, G. A., AND MOSES, A. J.: Kanamycin treatment of experimental infections in mice, *Ann. New York Acad. Sc.* 76:81-87, 1958.
6. HEWLITT, W. L., AND FINEGOLD, S. M.: Laboratory studies with kanamycin, *Ann. New York Acad. Sc.* 76:122, 1958.
7. GREEY, P. H., AND WIGHTMAN, K. J. R.: Kanamycin experience in selected cases, *Ann. New York Acad. Sc.* 76:122-128, 1958.
8. THURMAN, W. G., AND PLATOU, R. V.: Some experiences with kanamycin in the treatment of *Salmonella* and *Shigella* infections, *Ann. New York Acad. Sc.* 76:230-233, 1958.
9. KNIGHT, V.: The present staphylococcal problem, *Ann. New York Acad. Sc.* 76:274-276, 1958.
10. COHN, L., JR.: Kanamycin for bowel sterilization, *Ann. New York Acad. Sc.* 76:212-223, 1958.
11. ICHIKAWA, T.: Kanamycin in urinary infections, *Ann. New York Acad. Sc.* 76:242-264, 1958.
12. DUBE, A. H.: Kanamycin in the treatment of chronic urinary tract infections, *Ann. New York Acad. Sc.* 76:265-270, 1958.
13. MARMELL, M.; SHIDLOVSKY, B. A., AND PRIGOT, A.: Effect of carbomycin and erythromycin on the gram positive bacterial flora of the colon of man, *Harlem Hosp. Bull.* 8:24, 1955.
14. PRIGOT, A.; SHIDLOVSKY, B. A.; TURELL, R., AND MARMELL, M.: Presurgical preparation of the large bowel with neomycin and neomycin in combination with other antibiotics. I. Neomycin administered for various intervals and in combination with erythromycin. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, pp. 451-456.

# The Effect of Furazolidone Alone and in Combination with Neomycin on the Aerobic Intestinal Microflora of Man

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Military surgeons after World War II reported that the topical application of certain nitrofurans produced healing of infected wounds that had been recalcitrant to the sulfonamides and penicillin.<sup>1,2</sup> In vitro studies<sup>3-6</sup> of these compounds revealed powerful antimycotic properties and an antimicrobial range that included gram-positive and gram-negative bacteria, as well as some large viruses.

The development of soluble powders, solutions, and suppositories gradually extended the usefulness of the nitrofurans in dermatology, otolaryngology, ophthalmology, and in the therapy of vaginal and urethral infections in women. The peroral preparation of nitrofurantoin was found especially effective in the treatment of pyelonephritis, cystitis, and prostatitis.<sup>7</sup>

Furazolidone (N-5-nitro-2-furfurylidene-3-amino-2-oxazolidone) (Furoxone\*), a synthetic organic derivative of the nitrofuran series, was also considered appropriate for oral administration. Its pharmacology and toxicity have been well documented in the last few years. It has proved valuable to the poultry industry in the control of infections that were formerly economic catastrophes. The majority of these diseases originated with bacteria and fungal flora similar to those that may populate the human gastrointestinal tract.

Reports by MacLeod et al<sup>7</sup> and many other investigators have substantiated the antimicrobial, antiviral, and antimycotic potential of furazolidone in human infections. This compound is bacteriostatic against *Micrococcus pyogenes* var. *albus* and *aureus*, species of *Salmonella*, *Shigella*, *Klebsiella*, and *Vibrio*, as well as *Escherichia* and *Bacillus anthracis*. Streptococci in general are less sensitive than these groups of organisms.

Recently, Ponce de Leon<sup>8</sup> reported the cure of 25 of 30 children with acute diarrhea caused by *Salmonella*, *Shigella*, *Proteus*, and coliform species. This particularly stimulated our interest in a clinical survey utilizing furazolidone as an agent for antiseptics of the intestines as preparation for large bowel surgery.

The in vitro and in vivo spectrum peculiar to furazolidone suggested the possibility of reducing the number of staphylococcal colonies and those of yeasts and fungi found in the stool. Neomycin, on the other hand, is generally effective against the gram-negative organisms and, to a lesser degree, against the fecal streptococci. It was therefore postulated that the combination of furazolidone with neomycin might decrease significantly the total aerobic intestinal bacteria, as well as the yeasts and fungi.

This paper presents data accumulated on the effect of furazolidone alone in two dosage schedules, neomycin alone, and a combination of the two drugs on the intestinal microflora.

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This investigation was supported, in part, by a grant from the Eaton Laboratories, Norwich, N. Y.

\* The trade name of Eaton Laboratories for furazolidone is Furoxone.

TABLE I  
*Dosage Schedules of Furazolidone and Neomycin Employed for Intestinal Antisepsis*

Schedule	Number of patients	Medication	Total dosage, (Gm.)
1	5	Furazolidone, 200 mg. 4 times daily, orally for 1 day	0.8
2	5	Furazolidone, 400 mg. 4 times daily, orally for 1 day	1.6
3	5	Neomycin, 1 Gm. 4 times daily, orally for 1 day	4.0
4	5	Furazolidone, 400 mg., 4 times daily, orally for 1 day plus neomycin, 1 Gm., 4 times daily, orally for 1 day	$\left\{ \begin{array}{l} 4.0 \\ 1.6 \end{array} \right.$

#### MATERIALS AND METHODS

The patients selected were hospitalized for various conditions, usually traumatic in origin, and were free of any recent history of gastrointestinal pathology. For at least one week prior to the study, they had received no medication that might interfere with the interpretation of the bacteriological results. All subjects received the routine hospital diet, and no mechanical cleansing of the intestines was performed during the investigation.

Furazolidone, available in 100 mg. tablets for peroral use, was administered alone at two dosage levels and combined with neomycin in another, as listed in table I. The neomycin (Mycifradin sulfate\*) was in compressed tablets of 0.5 Gm.

The assay of aerobic microflora of fecal specimens recovered from these patients was based on serial dilutions of a given weight of wet stool in a sterile diluent. Aliquots of appropriate dilutions of stool suspension were then utilized to make both streak and pour plates before, during, and after medication. The colony numbers on various media were recorded and the organisms identified. These colony counts constituted an index of the aerobic microflora.

#### RESULTS

Pretreatment colony counts of organisms from the stools of patients under observation were compared with assays from specimens recovered 18 to 24 hours post-treatment. The numerical differences were calculated as percentage changes. The average of the percentage changes for each drug or the combination of both drugs is depicted in figure 1. The results of assays for the individual patients are shown in table II.

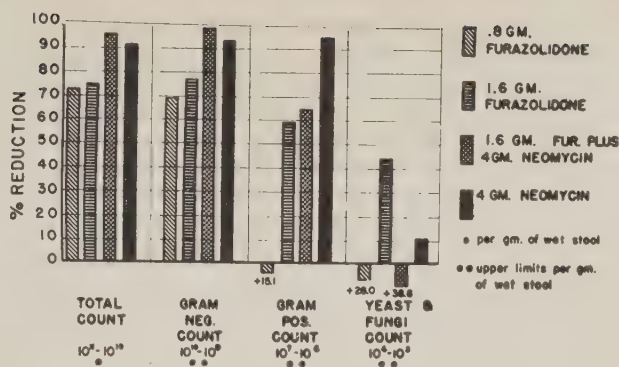
*Furazolidone.* Numerical reduction of intestinal microflora 18 to 24 hours after termination of treatment was found in the stools of patients treated with a total of 0.8 Gm. (schedule 1) and also of those patients who received 1.6 Gm. of furazolidone (schedule 2).

In the post-treatment stool specimens of the 0.8 Gm. group, 23.1 to 91.5 per cent reduction of the total bacterial count was observed, yielding an average reduction of 73.1 per cent. The gram-negative organisms were reduced 12.5 to 99 per cent, an average reduction of 69.9 per cent. The gram-positive bacteria and the yeasts and fungi increased in numbers, with an average increase of +15.1 and +28.0 per cent, respectively.

Furazolidone in the 1.6 Gm. total dosage produced greater and more consistent reduction in all groups of intestinal microflora. The total bacterial range of reduction was 12.8 to 94.9 per cent, with an average of 76.1 per cent. The gram-negative

\* The trade name of The Upjohn Co. for neomycin is Mycifradin sulfate.

FIG. 1. The effect of furazolidone alone and in combination with neomycin on the aerobic intestinal microflora of man is illustrated.



bacterial count decreased between 18.9 and 99 per cent, yielding an average reduction of 77.7 per cent. In the gram-positive bacterial count, decreases ranged from 22.7 to 91.2 per cent, averaging 59.9 per cent. Decreases in the yeasts and fungi were from 0 to 99.9 per cent, with an average of 44.4 per cent. Species of *Proteus* and *Pseudomonas* were evident in a number of post-treatment specimens throughout the study.

*Neomycin* (Schedule 3). A consistent and significant reduction of the total bacterial count was observed in stools of all patients receiving the antibiotic in a total dosage of 4 Gm. The decreases were from 93.1 to 99.0 per cent, producing an average reduction of 96.3 per cent, approximately 18 to 24 hours after the last dose. The numbers of aerobic gram-negative bacteria were reduced 97 to 99.9 per cent, the average decrease being 98.8 per cent. There was no evidence that either the *Proteus* or *Pseudomonas* species increased in numbers, although both were isolated along with other intestinal microflora.

TABLE II

Effect of Furazolidone and Neomycin on Intestinal Microflora, Per Cent Reduction in 18 to 24 Hours Post-Treatment Compared with Pretreatment Specimens

Medication and total dosage, Gm.	Patient	Total bacterial count	Gram-negative bacteria	Gram-positive bacteria	Yeasts and fungi
Furazolidone, 0.8	R. A.	-23.1	-12.5	-73.7	-34.8
	F. C.	-75.0	-65.9	+75.7	+99.9
	D. S.	-87.0	-85.0	+67.7	0
	Y. G.	-89.0	-99.0	+99.9	+99.9
	N. C.	-91.5	-87.0	-93.8	-25.0
	Average	-73.1	-69.9	+15.1	+28.0
Furazolidone, 1.6	R. C.	-12.8	-18.9	-22.7	-18.9
	T. B.	-93.9	-77.8	-91.2	-99.9
	R. F.	-94.9	-94.4	-57.4	-45.4
	M. B.	-87.3	-97.4	-48.7	-57.8
	N. L.	-91.6	-99.0	-79.8	0
	Average	-76.1	-77.5	-59.9	-44.4
Neomycin, 4	S. C.	-96.8	-99.9	-99.9	+30.1
	C. J.	-93.1	-99.9	-30.3	-75.0
	R. J.	-99.0	-97.0	-70.6	+79.2
	S. J.	-95.4	-98.0	-80.0	-15.4
	A. L.	-97.4	-98.8	-45.0	+20.0
	Average	-96.3	-98.8	-65.2	+38.8
Furazolidone, 1.6, plus neomycin, 4	N. W.	-92.1	-99.9	-95.4	-14.0
	W. S.	-92.1	-99.9	-93.7	-14.4
	G. R.	-81.7	-90.0	-89.6	-14.4
	H. Mc.	-99.9	-90.6	-99.9	0
	B. L.	-93.0	-89.0	-96.0	-12.0
	Average	-91.8	-93.8	-94.9	-10.9

Enterococci and staphylococci, which dominate the aerobic gram-positive intestinal population, were decreased inconsistently, 30.3 to 99.9 per cent, producing an average reduction of 65.2 per cent. Both of these species persisted throughout the study in all patients receiving neomycin.

Yeasts and fungi were either insignificantly reduced or increased. In 3 of the 5 patients, there was an increase of 20.0, 30.1, and 79.2 per cent, respectively, in colony counts above the pretreatment assays.

*Furazolidone and Neomycin (Schedule 4).* The combination of 1.6 Gm. of furazolidone with 4 Gm. of neomycin reduced the total bacterial count between 8.7 and 99.9 per cent, giving an average reduction of 91.8 per cent. This level is slightly less than that obtained with neomycin alone, where the reduction ranged between 93.1 and 99 per cent, with an average of 96.3 per cent. The effect of furazolidone and neomycin combined on the gram-negative bacteria was demonstrated in reductions between 89 and 99.9 per cent, with an average decrease of 93.8 per cent, which approximates the results with neomycin alone (97 to 99.9 per cent, average reduction of 98.8).

On aerobic gram-positive organisms the combined medication exerted a distinctly greater influence. The decreases ranged from 89.6 to 99.9 per cent, producing an average reduction percentage of 94.9.

Yeasts and fungus populations assayed in pretreatment stool specimens were reduced inconsistently with results ranging from 0 to 14.4 per cent, yielding an average decrease of 10.7 per cent as compared to the results with 800 mg. of furazolidone alone (0 to 99.9 per cent, average reduction percentage of 44.4).

#### DISCUSSION

During the past few years the surgical research laboratory of Harlem Hospital has conducted a screening program to determine what chemotherapeutic agent or combination of such agents will most effectively reduce the intestinal flora of man. These drugs may be employed to decrease peritoneal contamination by intestinal bacteria in cases where large bowel surgery is indicated. The criterion for significant reduction in our screening program is a decrease of 90 per cent or more in the number of colonies found in the pretreatment stool specimen.

The administration of furazolidone and neomycin orally to hospitalized patients has produced substantial reduction in intestinal flora for a limited period of time. However, the increase in microflora occurring subsequent to the 24 hour post-treatment reduction indicates that the intestinal contents are not completely sterilized by these drugs.

Both neomycin alone and the combination with furazolidone reduced total bacterial counts, and the gram-negative colonies were reduced significantly to approximately the same degree. Due to the limited number of patients involved and the margin of error in assaying stools, it is believed that differences in figures of less than 10 per cent cannot be considered statistically valid.

In reducing the aerobic gram-positive bacteria in the stool, it appears that combining furazolidone with neomycin enhances their inhibitory effect, since only this combination decreased this count to a significant degree.

Neither of the drugs used alone nor the combination appreciably influenced the yeasts and fungi.

It appears that the combination of furazolidone and neomycin would be more effective in bowel sterilization than either drug alone, since only the combined medi-

cation produced significant decreases in the total fecal bacterial colonies and the gram-negative and gram-positive aerobic flora.

#### SUMMARY

The effect of neomycin and furazolidone, each alone and in combination, on the aerobic microflora found in the human stool was studied.

Only the combination of furazolidone and neomycin produced a significant reduction (94.9 per cent) in aerobic gram-positive bacteria.

Both neomycin alone and the combination produced similar degrees of reduction in both the total counts and gram-negative counts 18 to 24 hours after completion of treatment.

The greatest reduction of yeasts and fungi (44.4 per cent) occurred with 400 mg. of furazolidone.

No toxic manifestations were observed in any of the 20 patients involved in this study.

#### CONCLUSION

In a program of screening antibacterial agents for intestinal antiseptics, data accumulated on furazolidone used alone indicate variable effects on the total bacterial population and on the gram-negative and gram-positive colonies. When furazolidone is employed in combination with neomycin, the inhibition of intestinal microflora is more consistent and is within the order of activity that we consider appropriate for presurgical preparation of the large bowel.

#### ACKNOWLEDGMENT

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#### BIBLIOGRAPHY

1. MCCOLLOUGH, N. C.: Treatment of infected war wounds with a nitrofurantoin, *Indust. Med.* 16:128, 1947.
2. SNYDER, M. L.; KIEHN, C. L., AND CHRISTOPHERSON, J. W.: Effectiveness of a nitrofurantoin in the treatment of infected wounds. I. Preliminary report, *Mil. Surgeon* 92:380, 1955.
3. YURCHENCO, J. A.; YURCHENCO, M. C., AND PIEPOLI, C. R.: Antimicrobial properties of furoxone (N-5-nitro-2-furfurylidene-2-amino-2-oxazolidone), *Antib. & Chemo.* 3:1035, 1953.
4. WARD, W. C.; PRYTHERCH, J. P., AND CRAMER, D. L.: The in vitro fungistatic action of some nitrofurantoin derivatives, *J. Am. Pharm. A. (Scient. Ed.)* 37:317, 1948.
5. ROGERS, G. S.; BELLOFF, G. B.; PAUL, M. F.; YURCHENCO, J. A., AND GEVER, G.: Furazolidone, a new antimicrobial nitrofurantoin: a review of laboratory and clinical data, *Antib. & Chemo.* 6:231, 1956.
6. RITTS, R. E., JR.; MAO, F. H., AND FAVOUR, C. B.: Sensitivity of clinically significant genitourinary tract pathogens to antimicrobial agents. In: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 774-782.
7. MACLEOD, P. F.; ROGERS, G. S., AND ANZLOWAR, B. R.: The nitrofurantoin in clinical medicine, *Internat. Rec. Med. & G. P. C.* 169:561, 1956.
8. PONCE DE LEON, E.: Furazolidone (Furoxone) in the treatment of the acute bacterial diarrheal syndrome, *AM & CT* 4:814, 1957.

# Studies on the Mechanism of Action of Kanamycin

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Kanamycin\* is a new antibiotic, isolated by Umezawa et al<sup>1</sup> from fermentation beers of an actinomycete, *Streptomyces kanamyceticus*. Chemically it is a water-soluble basic compound with hydroxyl and primary amine functional groups. Its structure was shown to be that of deoxystreptamine linked glycosidically to two amino sugars, 3-glucosamine and 6-glucosamine.<sup>2</sup>

Some of the microbiological properties of kanamycin have been previously described.<sup>3-5</sup> In the present work the bacteriological properties of the antibiotic have been further studied primarily from the point of view of attempting to shed some light on its mode of action.

## MATERIALS AND METHODS

*Cultures.* Most work was done with various strains of *Escherichia coli*. The kanamycin-sensitive strains used were ATCC 8739, PO 1495, K-12, and W-1485, the latter two being supplied by Lederberg. Resistant strains were obtained through serial passages in media containing increasing concentrations of kanamycin.

*Bacillus subtilis* ATCC 6633 and *Clostridium histolyticus* ATCC 6282 were received from the American Type Culture Collection in Washington, D. C. *Clostridium welchii* was an isolate from human infection.

The cultures other than clostridia were maintained on heart infusion agar or nutrient agar slants. Clostridia were maintained in thioglycollate broth. Routinely, 18 hour heart infusion or thioglycollate broth transfers were used for experiments.

*Media.* Various Difco or Baltimore Biological Laboratory prepared media were used, made up according to the manufacturers' instructions. The heart infusion plating medium was prepared from Difco heart infusion broth by addition of 2 per cent agar. Soft agars were prepared by addition of 0.2 or 0.5 per cent of agar. Defined media reported by Davis and Mingioli<sup>6</sup> and Witkin<sup>7</sup> were used where indicated. As these media contain phosphate and Davis' medium contains in addition citrate, material found to be antagonistic to kanamycin action, a barbiturate-glucose-salt medium was used in some instances. It contained, per liter, 5, 5-diethyl barbituric acid, 2.3 Gm., sodium diethyl barbiturate, 1.5 Gm., ammonium chloride, 1 Gm., hydrated magnesium sulfate, 0.7 Gm., potassium chloride, 0.1 Gm., and glucose, autoclaved separately, 5 Gm. The pH was adjusted before sterilization to pH 7.2 with hydrochloric acid.

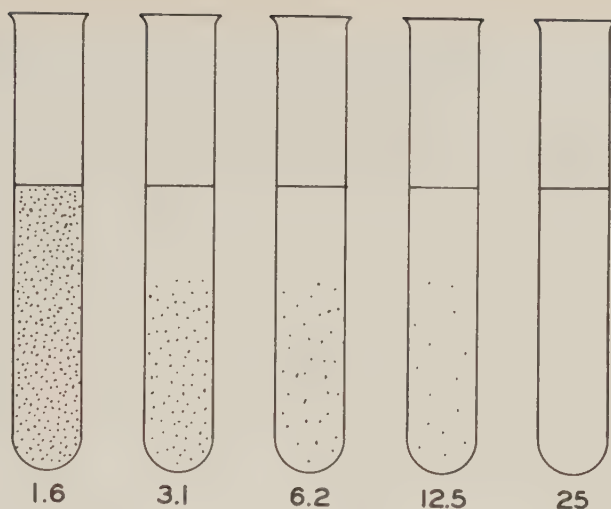
Dilutions, except as otherwise noted, were done in sterile 1 per cent saline.

*Methods.* Minimum inhibitory concentrations (MIC) were determined by the standard twofold serial transfer procedure. The overnight culture of the organism was diluted 1:10,000 (or 1:100 in case of poor growth) and 1 ml. portions were dispensed into a series of tubes. One ml. of a known concentration of a sterile kanamycin solution was introduced into the first tube, the contents mixed, 1 ml. of the mixture withdrawn and introduced into the second tube, and the procedure repeated. Tubes were incubated at 37 C. The MIC was recorded as the lowest concentration of the antibiotic that prevented growth.

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\* The trade name of Bristol Laboratories Inc. for kanamycin is Kantrex.

FIG. 1. Schematic representation of the inhibition of *Staphylococcus aureus* by kanamycin in soft agar. Figures represent  $\mu\text{g./ml.}$  kanamycin.



The soft agar technique of Heinemann et al<sup>8</sup> was used for determining the influence of oxygen tension.

Determination of the bactericidal action of kanamycin and of protective action of various compounds was patterned after experiments on antagonism and synergism.<sup>9</sup> A technique based on the auxanographic method was also used to determine the antagonism of various ingredients to kanamycin. Pure materials were added to filter paper discs, which were applied to the surface of seeded media containing sufficient kanamycin to prevent the bacteria from growing. Zones of growth around the discs indicated that the added material antagonized kanamycin's action.

## RESULTS

*Influence of Oxygen Tension.* Examination of the antibiotic spectrum revealed that the anaerobes and microaerophils like clostridia and lactobacilli were naturally resistant to kanamycin. In order to extend these observations, a culture of *Clostridium* that according to *Bergey's Manual*<sup>10</sup> is an anaerobe but that will grow scantily

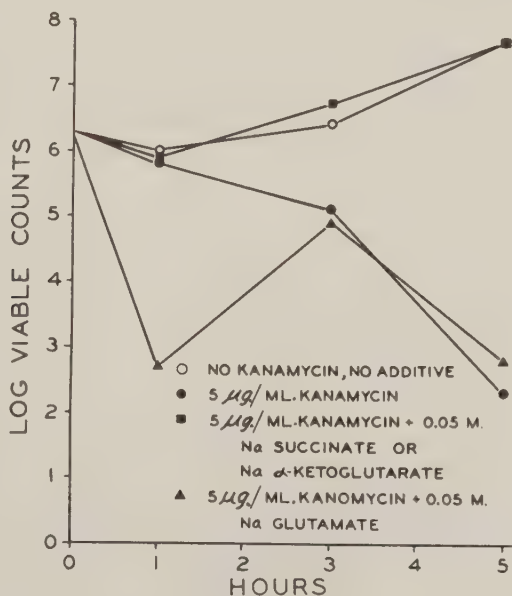


FIG. 2. Inhibition of kanamycin action on *Escherichia coli* by some dicarboxylic acids.

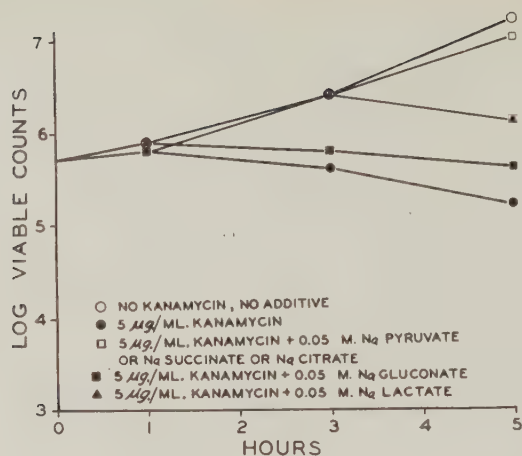


FIG. 3. Inhibition of kanamycin action on *Escherichia coli* by some acids in Witkin's M-9 medium.

under aerobic conditions, namely, *Clostridium histolyticus*, was compared with a strict anaerobe, *Clostridium welchii*. The MIC values in thioglycollate broth for these cultures were 8 and 250  $\mu$ g./ml., respectively, again indicating a correlation of sensitivity with aerobic respiratory potential.

This concept was examined further experimentally by growing *E. coli* in soft agar media. Under these conditions, the top portion of the tube is aerobic, the bottom anaerobic. Determination of the MIC for the strains PO 1495 and K-12 was made for both the top and the bottom of the tube. The results are schematically represented in figure 1 and indicate that the aerobic layer is inhibited at 4 to 16 times lower levels of the antibiotic than the anaerobic bottom layer.

*Effects of Tricarboxylic Acid Cycle Components and Related Compounds.* The influence of the addition of various components of the Krebs tricarboxylic acid cycle and related compounds was studied by measuring their effect on the rate of killing by kanamycin.

One of the typical experiments is represented in figure 2. *E. coli*, strain ATCC 8739, was grown in Davis' synthetic medium in the absence of supplements, in the presence of kanamycin alone, and in the presence of kanamycin and either sodium succinate, sodium  $\alpha$ -ketoglutarate, or sodium glutamate. As can be seen, sodium succinate and sodium  $\alpha$ -ketoglutarate reversed the action of the antibiotic, while sodium glutamate did not. After these results were found, it was decided to repeat the experiments with other Krebs cycle acids. In these experiments, a different medium was used because Davis' medium contained one of the acids under

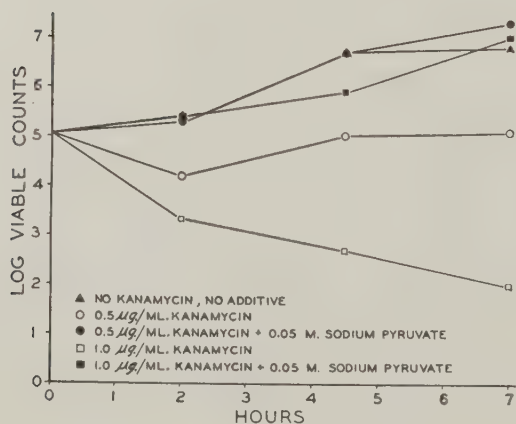


FIG. 4. Inhibition of kanamycin action on *Bacillus subtilis* by pyruvate in heart infusion broth.

TABLE I  
Influence of Media Ingredients on Inhibition of *E. coli* by Kanamycin

Medium	MIC ( $\mu\text{g./ml.}$ ) with following additives				
	None	0.5% sodium chloride	2.5% heart infusion broth solids	0.1% phosphate buffer	0.5% yeast extract
Barbiturate	0.8	—	12.5	3.1	0.8
M-9	6.2	—	25	—	—
Davis'	1.5	—	12.5	—	—
Nutrient broth	<0.2	3.1	6.2	—	—
Heart infusion broth	6.2	—	—	—	—

study, namely, citric acid. The results of the experiments with sodium pyruvate, succinate, citrate, and gluconate in Witkin's M-9 medium are shown in figure 3. Again, the acids of the tricarboxylic acid cycle, pyruvate included, reversed the action of the antibiotic. A related acid, lactic, also reversed but to a lesser extent, and gluconic acid did not reverse at all. Other experiments showed that malic, oxalacetic, cis-aconitic, and isocitric acids all exhibited this activity. These reversals require a relatively high concentration of the active compounds, namely, 0.05 M. Concentrations of 0.005 M. were without effect. Other organisms and media were tried with similar effect. As an example, the reversal of *Bacillus subtilis* inhibition by pyruvate is shown in figure 4.

Two facts emerge from these experiments: (1) cells with an aerobic respiratory pathway are more easily affected than those with an anaerobic one; (2) the components of the tricarboxylic acid cycle can protect against kanamycin's action. The data are consistent with the idea that this antibiotic interferes with the normal operation of the tricarboxylic acid cycle resulting in a deficiency of energy or intermediates obtained from the operation of the cycle.

In an attempt to substantiate this hypothesis, experiments were carried out studying cross resistance between kanamycin and azide. It was thought that kanamycin-resistant cells might acquire a biochemical shunt that would make them resistant to the action of compounds like azide, which is known to interfere with oxidative respiration.

TABLE II  
Sensitivity to Kanamycin or to Sodium Azide of Mutants of *B. subtilis* and *E. coli* Made Resistant to One or the Other Agent

Organism	Isolate	Resistance developed to	MIC	
			Kanamycin, $\mu\text{g./ml.}$	Azide, $\text{mg./ml.}$
<i>B. subtilis</i>	Parent	—	0.16	0.31
	1	Kanamycin	31	0.012
	2	Kanamycin	16	0.012
	3	Azide	$\leq 0.08$	10.0
	4	Azide	8.0	2.5
<i>E. coli</i>	Parent	—	4.0	0.16
	5	Kanamycin	500	0.31
	6	Kanamycin	250	0.31
	7	Azide	1.25	2.5
	8	Azide	1.25	1.25
	9	Azide	1.25	5.0
	10	Azide	5.0	2.5
	11	Azide	2.5	2.5

Cells of a facultative anaerobe, *E. coli*, and of an obligate aerobe, *B. subtilis*, were made resistant either to kanamycin or to sodium azide. The resistant cultures were plated, single colonies picked, and the resistance to both agents determined simultaneously for each colony. As can be seen in table I, no definite evidence for cross resistance between azide and kanamycin was noted. However, a collateral sensitivity was observed in some cases. *B. subtilis* cells made resistant to kanamycin are more sensitive to azide than the parent culture. This was not the case with *E. coli*. The collateral sensitivity of the *B. subtilis* might indicate that both materials act in the same general area of biochemical reactions but at different reaction sites, again implicating the aerobic respiratory system as a prime target of kanamycin.

*Effects of Other Media Ingredients.* Other ingredients were also shown to affect kanamycin's activity. This is reflected in the different MIC obtained with different media. Table II shows the influence of various additives on the MIC obtained with various media. Large effects are observed with sodium chloride, phosphate buffer, and heart infusion solids.

Experiments that were conducted using the auxanographic technique, although rather qualitative in nature, gave similar indications. Various media constituents, such as Difco peptone, N-Z case, and heart infusion solid, were antagonistic to the action of kanamycin. In all cases, heart infusion solid was the most active of the kanamycin antagonists. Whole human serum showed no protective action.

In a previous paper,<sup>5</sup> it was shown that kanamycin-treated cells are osmotically fragile. If they are diluted in water, they are killed, but if they are diluted in saline, they will survive. This is true for concentrations of kanamycin just below the bactericidal level. It is probable therefore that the influence of saline in these experiments is protective. The osmotically fragile cells can survive in a medium containing this constituent. Whether the other ingredients also act through this mechanism remains to be determined.

## DISCUSSION

The spectrum of activity of kanamycin suggested in itself that the mode of action of kanamycin was related to aerobic respiration in some way. The experiments showing that the Krebs cycle components can reverse the bactericidal action of kanamycin point to this enzymatic complex as the prime target. This does not imply that the interference is a simple enzymatic one, such as competition with one of the intermediates. A more profound effect such as on the mitochondrial structure may be the basis of the inhibition. One would suspect that effective antibiotic agents operate on a higher level of organization in the cell than on a single biochemical reaction.

The observations that kanamycin-treated cells are osmotically fragile does not contradict this hypothesis. The two phenomena may be related by the fact that interference with the Krebs cycle may lead to faulty synthesis of precursors or to a breakdown in the normal energetics that may be needed for cell permeability.

## SUMMARY

Kanamycin is more active under aerobic than anaerobic conditions. Acids of the Krebs cycle are antagonistic to the action of kanamycin, as are some salts and complex organic nutrients. An increased osmotic fragility is also observed.

The major biochemical lesion produced by kanamycin appears to be associated

with the oxidative respiratory pathway. This apparently results in an increased osmotic fragility of the affected cells.

#### BIBLIOGRAPHY

1. UMEZAWA, H.; UEDA, M.; MAEDA, K.; YAGASHITA, K.; KONDO, S.; OKAMI, Y.; UTAHARA, R.; OSATO, Y.; NITTA, K., AND TAKEUCHI, T.: Production and isolation of a new antibiotic, kanamycin, *J. Antib., ser. A* 10:181-188, 1957.
2. CRON, M. J.; EVANS, D. L.; PALERMITI, F. M.; WHITEHEAD, D. F., AND HOOPER, I. R.: Kanamycin V. The structure of kanosamine, *J. Am. Chem. Soc.*, 1958. In press.
3. TAKEUCHI, T.; HIKIJI, T.; NITTA, K.; YAMAZAKI, S.; ABE, S.; TAKAYAMA, H., AND UMEZAWA, H.: Biological studies on kanamycin, *J. Antib., ser. A* 10:107-114, 1957.
4. GOUREVITCH, A.; HUNT, G. A., AND LEIN, J.: Antibacterial activity of kanamycin, *Antib. & Chemo.* 8:149-159, 1958.
5. GOUREVITCH, A.; ROSSOMANO, V. Z.; PUGLISI, T. A.; TYNDA, J. M., AND LEIN, J.: Microbiological studies with kanamycin, *Ann. New York Acad. Sc.* 76:31, 1958.
6. DAVIS, B. D., AND MINGIOLI, E. S.: Mutants of *Escherichia coli* requiring methionine or vitamin B-12, *J. Bact.* 60:17-28, 1950.
7. WITKIN, E. M.: Mutations in *Escherichia coli* induced by chemical agents, Cold Spring Harbor Symposia on Quantitative Biology 12:256-269, 1947.
8. HEINEMANN, B.; HUNT, G. A., AND MISIEK, M.: A microbiologic procedure for presumptive identification of certain antibiotics. In: Antibiotics Annual 1955-1956, New York, Medical Encyclopedia, Inc., 1956, pp. 391-395.
9. GUNNISON, J. B.; JAWETZ, E., AND COLEMAN, V. R.: The effect of combinations of antibiotics on Enterococci *in vitro*, *J. Lab. & Clin. Med.* 36:900-911, 1951.
10. Bergey's Manual of Determinative Bacteriology, ed. 7, Baltimore, The Williams & Wilkins Co., 1957.

# A Modified Two-Point Turbidimetric Assay for Kanamycin

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A turbidimetric assay for kanamycin\* A and B<sup>2</sup> has been developed using a modified two-point assay method, with a simplified method of calculation. The turbidimetric assay was chosen because it gave a valid measurement of both kanamycin A and B, singly or in combination. A plate agar-diffusion method was shown to be invalid for kanamycin B or mixtures of A and B, due to a difference in rates of diffusion.

The assay design is based on the theory of parallel line assays<sup>3,4</sup> with modifications to correct for significant sources of error. Calculations are simplified so that a readily derived factor is multiplied by the difference in level between unknown and standard to give the deviation in terms of per cent of standard.

## MATERIALS AND METHODS

The medium employed for this assay is antibiotic assay broth (Baltimore Biological Laboratories no. 01-171) made up to 85 per cent of recommended strength. *Staphylococcus aureus* 209 P is used as the test organism. The standard is a kanamycin A base, with a theoretical potency of 960  $\mu\text{g.}/\text{mg.}$

Standard dilutions are made up to 1.6, 2.0, and 2.4  $\mu\text{g.}/\text{ml.}$  in 0.1M, pH 8.0 phosphate buffer. Unknown samples are diluted to estimated concentrations of 1.6 and 2.4  $\mu\text{g.}/\text{ml.}$  in buffer. Nine ml. of inoculated media is added to both the standard and unknown tubes, and all tubes are incubated at 37 C. in a circulating water bath. The tubes are removed from the bath and read when the 1.6  $\mu\text{g.}/\text{ml.}$  standard tubes read 0.250 optical density on a Bausch and Lomb Spectronic 20 colorimeter set at 530  $\text{m}\mu$ .

Three tubes of unknown are incubated at each level of dilution. Three tubes of standard, at each level of standard, are incubated in each rack of unknowns. No more than 27 tubes are incubated in a rack with a capacity of 40 tubes, the tubes being arranged so as to allow for circulation of water throughout the rack.

Potencies are then calculated as follows: the difference between the sum of the readings of the three standard tubes diluted at 1.6  $\mu\text{g.}/\text{ml.}$  and the sum of the readings of the three standard tubes diluted at 2.4  $\mu\text{g.}/\text{mg.}$  is calculated for each set of standards. The difference between the sum of the readings of the three tubes at the two levels of each unknown is also calculated. In order to minimize the error in assays due to errors in the slope computation, these differences may be averaged for both unknowns and standards for six racks read in order. No differences that exceed a range limit of 0.015 for from two to four sets of readings or a range limit of 0.020 for five or more sets should be averaged. If the high ranges are caused by differences between racks, a smaller group of racks or single racks should be used in calculating an average difference. If single sets of unknowns are out of range, these particular assays are invalid and should be repeated.

The slope factor for calculations is determined from table I, using the average differences calculated previously. The potency of an unknown, in terms of deviation from standard, in per cent of standard, may then be calculated by multiplying the

\* The trade name of Bristol Laboratories Inc. for kanamycin is Kantrex.

TABLE I  
*Factors for Potency Calculation, Kanamycin Turbidimetric Assay*

Average difference	Factor (f)	Average difference	Factor (f)
0.030	4000	0.145	830
0.035	3400	0.150	800
0.040	3000	0.155	770
0.045	2700	0.160	750
0.050	2400	0.165	730
0.055	2200	0.170	710
0.060	2000	0.175	690
0.065	1800	0.180	670
0.070	1700	0.185	650
0.075	1600	0.190	630
0.080	1500	0.195	620
0.085	1400	0.200	600
0.090	1300	0.205	590
0.095	1300	0.210	570
0.100	1200	0.215	560
0.105	1140	0.220	550
0.110	1090	0.225	530
0.115	1040	0.230	520
0.120	1000	0.235	510
0.125	960	0.240	500
0.130	920	0.245	490
0.135	890	0.250	480
0.140	860		

factor by the difference between the average of the standard readings for the rack in which the unknown was incubated and the average of the six unknown tube readings.

#### EXPERIMENTAL STUDIES

*Choice of Method.* An agar-diffusion method, using *Bacillus subtilis* (ATCC 6633), was also used experimentally for the assay of kanamycin. When samples of high kanamycin B content were assayed by both methods, it was found that a significant difference in assay level existed, even though both methods utilized the

TABLE II  
*Response of B. subtilis Agar-Diffusion Assay to Kanamycins A and B*

	Kanamycin concentration, $\mu\text{g./ml.}$				
	0.7	0.9	1.1	3.0	5.0
	(zone sizes in mm. average of eight zones)				
Kanamycin A	14.1	14.9	15.8	19.3	20.3
Kanamycin B	16.3	16.4	17.4	18.9	20.4
Source	Statistical analysis				F
	D.F.	S.S.	M.S.		
Level between A and B	1	21.47	21.47		82.6*
Regression (log-dose)	1	287.70	287.70		110.7*
Parallelism	1	16.28	16.28		62.6*
Linearity	6	34.65	5.78		22.2*
Between dose	9	360.10	40.01		153.9*
Within dose	70	18.39	0.26		
Total	79	378.49			

\* Significant at  $P = 0.001$ .

TABLE III

*Response of Staph. aureus Agar-Diffusion Assay to Kanamycins A and B*

	Kanamycin concentration, $\mu\text{g./ml.}$				
	0.7	0.9	1.1	3.0	5.0
	(zone sizes in mm. average of eight zones)				
Kanamycin A	12.7	13.4	14.0	16.9	18.7
Kanamycin B	14.5	15.0	15.8	18.2	18.8

Source	Statistical analysis			
	D.F.	S.S.	M.S.	F
Level between A and B	1	34.58	34.58	494.0*
Regression (log-dose)	1	321.08	321.08	4586.9*
Parallelism	1	5.89	5.89	84.1*
Linearity	6	2.97	0.49	7.0*
Between dose	9	364.52	40.50	578.6*
Within dose	70	4.66	0.07	
Total	79	369.18		

\* Significant at  $P = 0.001$ .

same kanamycin A base standard, and results agreed on samples of high kanamycin A content.

It was found that kanamycin B gave a significantly different slope from that of the kanamycin A standard when assayed by the agar diffusion method (table II). Parallel curves were obtained by the turbidimetric method with a kanamycin A standard being compared to samples of kanamycin B and mixtures of kanamycins A and B (table IV).

To check for a possible difference in sensitivity to kanamycin B between organisms, the *Staph. aureus* that was used in the turbidimetric assay was used to repeat the experiment on the plate-diffusion assay. A significant difference in slope between the two types of kanamycin was again obtained (table III). Since the possibility of difference in organism sensitivity was eliminated, and kanamycin B showed a higher relative potency at lower concentrations, it was concluded that

TABLE IV

*Response of Staph. aureus Turbidimetric Assay to Kanamycins A and B*

	Kanamycin concentration, $\mu\text{g./ml.}$		
	0.15	0.20	0.25
	(optical density, averages of six tubes)		
Kanamycin A	0.318	0.290	0.270
Kanamycin B	0.293	0.268	0.243

Source	Statistical analysis			
	D.F.	S.S.	M.S.	F
Level between A and B	1	0.005625	0.005625	181.5*
Regression (linear)	1	0.014259	0.014259	4599.6*
Parallelism	1	0.000001	0.000001	N.S.
Linearity	2	0.000070	0.000035	N.S.
Between dose	5	0.019955	0.003991	128.7*
Within dose	30	0.000937	0.000031	
Total	35	0.020892		

\* Significant at  $P = 0.001$ .

TABLE V

*Changes in Slope Due to Media Dilution, Kanamycin Turbidimetric Assay*

Dilution level (per cent of recommended strength)	Dosage level, $\mu\text{g.}/\text{ml.}$			Slope
	0.05 (optical density, averages of 12 tubes)	0.10	0.15	
100	0.270	0.254	0.241	-0.290
85	0.264	0.213	0.157	-1.070
75	0.251	0.232	0.216	-0.370

the invalidity of the plate method for kanamycin B or mixtures of A and B was due to differences in rates of diffusion between the two compounds.

It was also concluded that the plate diffusion assay was invalid for possible mixtures of the two types and should only be used for blood level assays of high kanamycin A content, where it possessed other advantages over the turbidimetric method.

*Choice of Media and Organism.* The turbidimetric assay was run experimentally with antibiotic assay broth (Baltimore Biological Laboratories 01-171) at recommended strength. In an effort to increase sensitivity, the medium was further diluted, and it was found that a more sensitive assay was possible at 85 per cent of recommended strength. Further dilutions of media changed the characteristics of the dosage-optical density slope; therefore the assay was standardized at 85 per cent (table V).

Twelve organisms were screened for growth characteristics and sensitivity, with *Staph. aureus* showing the best characteristics for the assay.

*Choice of Assay Design and Development of Calculation Method.* The kanamycin assay is at a low level of sensitivity as compared with the turbidimetric assay of tetracycline, for example, so that the normal errors made in a standard curve technique resulted in errors of at least 14 per cent under good conditions, with some replicate assays across days showing errors as high as 25 per cent.

The sources of error in the assay were studied by placing a set of standards in each assay rack and comparing these standards for differences in level and in slope between racks. It was found that a difference in level of optical density occurred between racks, but that the slope remained constant across reasonable differences in level, so that slope data might be pooled to minimize this source of error.

An assay design utilizing three levels of standard and two levels of unknown

TABLE VI

*Typical Comparison of Standard Tubes across Racks\**

Source	Turbidimetric kanamycin assay			
	D.F.	S.S.	M.S.	F
Level between racks	7	0.006216	0.000888	17.4†
Regression (linear)	1	0.018802	0.018802	361.5†
Parallelism (between racks)	7	0.000598	0.000085	1.7 N.S.
Linearity	8	0.000417	0.000052	1.0 N.S.
Between dose	23	0.026033	0.001131	22.2†
Within dose	48	0.002450	0.000051	
Total	71	0.028483		

\* Study across eight racks, three tubes of standard at each of three levels/rack.

† Significant at  $P = 0.001$ .

was then used, so as to establish a level of standard from each rack and make the best possible estimate of the slope from both unknown and standard readings.

Since the relationship between dosage and optical density has been shown to be linear, it was possible to simplify the potency calculations so that they were practical on a desk calculator.

Dosage levels were set up at even intervals so that the slope became a direct function of the total difference in reading between the 1.6 and 2.4  $\mu\text{g./ml.}$  levels. Since the Spectronic 20 can be normally read to the nearest 0.005, tables of factors were calculated in increments of 0.005 units of optical density. These factors were calculated by determining the slope by the method of least squares, then dividing this slope into 2.0  $\mu\text{g./ml.}$ , or the center point of the standard curve, so

$$\text{that the potency equation:}^3 M = \bar{x}_s - \bar{x}_t - \frac{(\bar{y}_s - \bar{y}_t)}{(b)}$$

where:  $M$  = estimate of potency,

$\bar{x}_s$  = average dosage level of standard,

$\bar{x}_t$  = average dosage level of unknown,

$\bar{y}_s$  = average optical density of standard,

$\bar{y}_t$  = average optical density of unknown,

simplifies to:  $M = f(\bar{y}_s - \bar{y}_t)$

where  $M$  is the potency in terms of deviation from standard as per cent of standard.

It will be noted that even though a negative slope exists, factors are used as positive multipliers, and the subtraction of unknown average density from that of the standard will thus give a negative deviation when a higher reading exists for the unknown, thus indicating a lower potency than the standard.

Three levels of standard were retained so that the linearity may be checked if necessary, and a larger number of standard tubes contributes to the average optical density of the standard.

This method does not enable a routine use of all validity checks that are possible with multiple point assays. The range limits for differences before pooling for use in factor determination serve as a partial check to eliminate invalid assays since significant deviations in slope will result from dilution errors or levels of dilution outside the linear portion of the curve.

*Precision of the Assay.* Since the error in this type of assay is a function of difference in level between unknown dilutions and the estimated concentrations, the error factor will vary with the precision of the estimate of unknown potencies. Calculations based on the residual error of this design and maximum deviations in unknown levels from standards in normal assay operations show that the maximum error should be less than 9 per cent at the 95 per cent confidence level.

A control chart based on the assay of known samples, set up at three dilutions and submitted as unknowns at random for assay, has shown that this level of error has not been exceeded since this procedure was introduced.

Variance analysis of a series of crude kanamycins and a series of finished kanamycin sulfates assayed experimentally across five days showed a relative error in each case of slightly less than 8 per cent at the 95 per cent confidence level. A recent study of assays on kanamycin sulfate finished bulks by Teegarden of our statistical division showed that the standard error across several months was 3.24 per cent for a three day assay at the 95 per cent confidence level.

#### SUMMARY

A turbidimetric assay for the routine determination of potency of kanamycin A

and B, singly or in combination, has been presented. A plate agar-diffusion method has been shown to be invalid for kanamycin B or combinations of B and A.

An assay design and method of calculation have been presented that minimized the errors in an assay with relatively poor sensitivity and furnished some validity checks.

#### BIBLIOGRAPHY

1. GAVIN, J. J.: Microbiological process report, analytical microbiology. III. Turbidimetric methods, *Appl. Microbiol.* 5: no. 4, 235-243, 1957.
2. UMEZAWA, H.; UEDA, M.; MAEDA, K.; YAGASHITA, K.; KONDO, S.; OKAMI, Y.; UTAHARA, R.; OSATO, Y.; NITTA, K., AND TAKEUCHI, T.: Production and isolation of a new antibiotic, kanamycin, *J. Antib. ser. A* 10:181-188, 1957.
3. FINNEY, D. J.: *Statistical Method in Biological Assay*, New York, Hafner Publishing Company, 1952.
4. EMMENS, C. W.: *Principles of Biological Assay*, London, Chapman and Hall, Ltd., 1948.

# A Resin Chromatographic Analysis for Kanamycin Mixtures

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Crude kanamycin isolated from fermentation broths of *Streptomyces kanamyceticus* by a cation-exchange resin process similar to that described by Umezawa and co-workers<sup>1,2</sup> was a mixture of several biologically active components. Paper strip chromatograms of the mixture developed with the *n*-butanol-water-2 per cent *p*-toluene sulfonic acid system indicated a complete separation of the major component, kanamycin A, from the other materials, but an estimation of the amount of each component by means of densitometer measurements on the chromatograms was generally unsatisfactory. Bioautographs were similarly unreliable.

Recrystallization of the mono sulfates was generally unsatisfactory for separating kanamycin A from kanamycin B,<sup>3,4</sup> because of mixed crystal formation as indicated by a roentgenographic diffraction pattern. Likewise, solubility analysis of the mono sulfate as a means of estimating the quantity of impurities was unreliable, because of solid solution formation and complications arising from the marked effect of excess sulfate ions. A complete separation of the A component was obtained by repeated recrystallization of the mixed kanamycin bases, but the recovery was quite low.

A chemical assay, which depended on the generation of a furfural-like substance, was used for the determination of kanamycin A since kanamycin B did not respond to this test. Biological assay methods determined both. The differential use of these assays to determine the nonkanamycin A materials was slow, tedious, and not as accurate as desired. Because kanamycin B was shown to be more toxic than the A component, a reliable quantitative method therefore was needed for determining the amount of kanamycin B in kanamycin preparations.

In using anion-exchange resins for the removal of color from kanamycin solutions, some retention of the antibiotic by strong base resins was observed. Further investigations revealed that porous, strongly basic anion-exchange resins with quaternary amine functional groups could be used at low loadings to separate kanamycin A from kanamycin B. Of these, Dowex 1-x2 resin was found satisfactory for the chromatographic analysis of kanamycin mixtures.

## MATERIALS AND METHODS

*Preparation of Chromatographic Column.* The chromatographic column used for most of this work was a four foot section of one inch internal diameter flanged Pyrex pipe mounted in a perpendicular position and coupled on the bottom end to a flanged Ultramax valve assembly containing a glass filter disc. A separatory funnel was used to maintain a head of water. The effluent stream was diverted through a flow meter and an inline conductivity cell (cell constant 2) to a fraction cutter. The conductivity cell was connected through a Wheatstone bridge and rectifier assembly to a 10 millivolt full range Varian strip-chart recorder (model G-11A). Limits of resistivity were set on the recording chart by means of a Heath standard resistance box.

Fifty to 100 mesh Dowex 1-x2 resin (chloride cycle) was backwashed free of fines before being converted to the hydroxyl cycle by column-wise washing with 2 resin volumes of 1 per cent sodium sulfate solution and 2 resin volumes of 10 per cent sodium hydroxide solution according to the directions of the manufacturer.

Because the resin shrinks and swells considerably, these operations were handled most easily in a large diameter column. The resin was washed free of alkali before loading into the chromatographic column.

The column was packed so that the resin did not entrap air bubbles, and there was a continuous column of liquid from the top of the column to the fraction collector. This was easily accomplished by filling the well at the bottom of the column and the column itself half full of water by backwashing. A thin water slurry of resin, from which all air bubbles were removed by stirring, was poured slowly into the column as water was drained from the bottom. The resin settled slowly, and as soon as the bed was of the desired depth, the column was ready for use. All connecting rubber tubing, the flow meter, and conductivity cell were filled with water before attaching to the bottom of the column.

A column prepared in this manner conveniently held 400 ml. of resin in a bed depth of 30 inches and would chromatograph as much as 10 Gm. of crude kanamycin. Most of the experience has been with columns of this size. However, for smaller samples proportionately smaller columns and volumes of resin would be used.

*Chromatographic Feed Solutions.* The crude kanamycin feed solution charged to the Dowex 1-x2 column may vary greatly in composition and concentration. Very impure materials obtained from the various steps in the resin isolation process as well as crude salts may be used. In order to avoid possible poisoning of the resin by highly colored solutions or partial conversion of the resin to the salt form by kanamycin salts, generally the resin should be cleaned and regenerated frequently. This inconvenience may be avoided by using Amberlite IRA-401 resin on the hydroxyl cycle to prepare decolorized kanamycin base solutions. With this type of charge, the Dowex 1-x2 column has been used repeatedly without diminution of efficiency. Although decolorized feed solutions of 5 to 50 per cent solids have been successfully chromatographed, most of the experience has been with 25 per cent solutions.

*Chromatographic Procedure.* The limits of resistivity for the Varian chart recording were set by means of a Heath standard resistance box between 6000 and 45,000 ohm-cm. Water of 45,000 ohm-cm. resistivity or greater must be used for developing the chromatogram and for washing the column preparatory to charging the feed solution.

A column containing 400 ml. of Dowex 1-x2 resin was drained to bed level and a sample of 1 to 10 Gm. of kanamycin as a 25 per cent feed solution was carefully charged to the column with a pipette. The effluent flow rate was regulated to 3.5 ml./minute. Chromatographic separation was not obtained at double this flow rate, that is, at half the contact time. After the charge had percolated into the resin, two or three 10 ml. volumes of water were placed on top of the resin and washed into the bed before a head of water was built up. Although aqueous solutions of methanol or acetone have been used to develop the chromatogram, water was found most satisfactory.

Fifty ml. portions of the eluate were collected by an automatic fraction cutter until the steadily increasing resistivity approached 45,000 ohm-cm. at the end of the elution of kanamycin A.

*Assays.* Whenever the composition of the column cuts was checked by paper chromatography, channeled Whatman no. 1 paper, 53 cm. in length, was used. Twenty  $\mu\text{g.}$  of solids was spotted to the papers that were dyed after development, while 5  $\mu\text{g.}$  was spotted to the papers placed on the bioautograph plates. All the

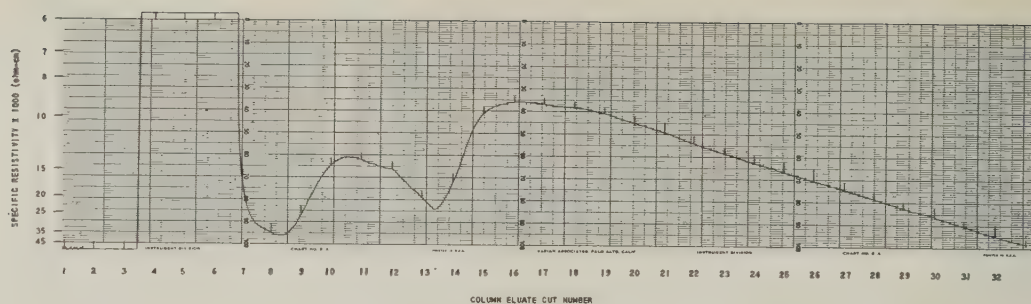


FIG. 1. Tracing of resistivity of eluate from a Dowex 1-x2 resin chromatographic separation of crude kanamycin mixture. Forerun in cuts 1-8, kanamycin B in cuts 8-12, mixture in cut 13, and kanamycin A in cuts 14-32. Kanamycin C appears as a shoulder at cut 12.

chromatograms were developed by the descending technique with Peterson and Reinecke's<sup>5</sup> *n*-butanol-water-2 per cent *p*-toluene sulfonic acid system. The chromatograms shown in figures 3 and 4 were developed for 20 hours, the one in figure 5 for 40 hours, and the one in figure 6 for 48 hours.

The dye used to locate the position of the kanamycins on the paper chromatograms, commonly called "chromato red," was made by coupling diazotized parosaniline with 1-naphthol-4-sulfonic acid.<sup>6</sup> The developed strips were dried thoroughly at room or higher temperatures before being dipped into a 0.1 to 0.5 per cent aqueous solution of this dye. The stained strips were hung vertically, and the excess dye was removed by several washes alternately with warm water and methanol. Prolonged washing diminished the intensity of the kanamycin C spot more rapidly than the A and B spots. The kanamycins were precipitated in red zones on the paper.

The chemical assay is based on the generation of a furfural-like material by sulfuric acid. A solution containing approximately 1 mg. of kanamycin base, 6 ml. of water, and 4 ml. of concentrated sulfuric acid was thoroughly mixed. One half of this sample was held in an ice bath and used as a blank in the spectrophotometer, while the other half was heated in a covered tube in a boiling water bath for one hour. Both samples were cooled to room temperature before a reading was made at 2800 Å. with a Beckman spectrophotometer (model DU). The reading of the unknown was compared with a standard.

The bioassays were determined by the standard cup assay method with *Bacillus subtilis*. The bioautograph plates were prepared with agar containing 0.00333 per cent triphenyltetrazolium chloride and seeded with *Escherichia coli* W according to the method of Stapley.<sup>7</sup> The individual channels were placed on the surface of the agar and incubated 18 hours at 25 C.

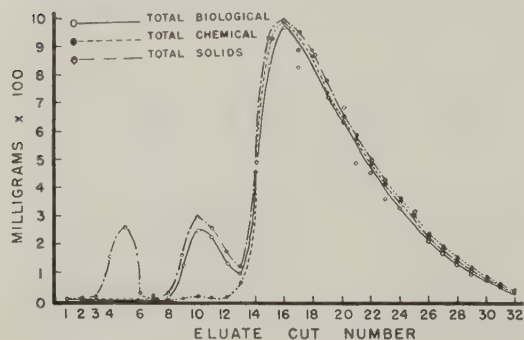
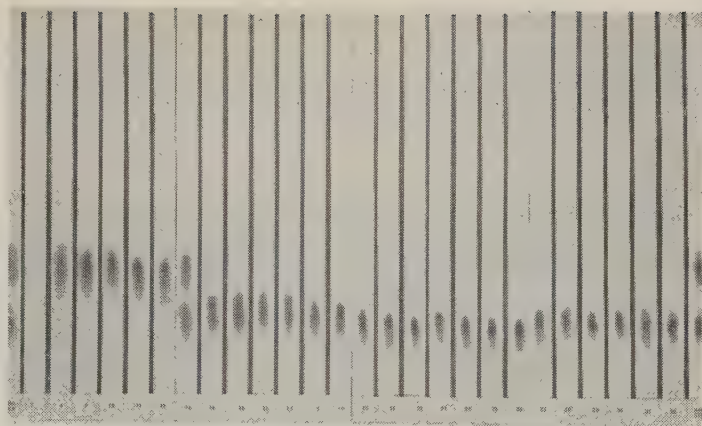


FIG. 2. Total solids, total chemical activity, and total bioactivity of cuts of eluate taken from a Dowex 1-x2 resin chromatographic separation of a crude kanamycin mixture. Forerun in cuts 1-8, kanamycin B in cuts 8-13, kanamycin A in cuts 13-32.

FIG. 3. The chromatographic separation of a crude kanamycin mixture on a Dowex 1-x2 resin column verified with chromatographic red dyed paper chromatograms. Kanamycin A  $R_f$  0.13–0.18 and kanamycin B  $R_f$  0.26–0.28. The channel paper strips read from left to right: Feed solution, 7–32, A plus B standard.



## RESULTS AND DISCUSSION

Figure 1 is a tracing of the eluate resistivity for a typical chromatogram. The cuts, numbered on the horizontal axis, were automatically registered on the chart by the recorder, which was quite sensitive to the electrical impulse that activated the fraction cutter. The specific resistivities are plotted on the vertical axis.

A column void of water of high resistivity equal to 0.3 resin volume was collected in cuts 1 to 3. This was followed by 0.4 resin volume of eluate in cuts 4 to 6 of very low resistivity due to small amounts of sodium hydroxide. The resistivity of the eluate increased abruptly through cut 7 to 35,000 ohm-cm. Kanamycin B was eluted in 0.7 resin volume in cuts 8 to 12, together with a small amount of another biologically active component, designated kanamycin C, which overlapped the end of the B curve. The shoulder at cut 12 indicated the presence of kanamycin C. Cut 13 was a mixture. The resistivity again reached a maximum, approximately 25,000 ohm-cm. between the A and B curves in cut 13. Kanamycin A was eluted in the following 2.5 resin volumes in cuts 14 to 32. The recovery of solids from this column was essentially quantitative. It appears likely that the kanamycins were adsorbed on the resin since ion exchange was not possible. The shape of the elution curve suggests adsorption chromatography.

The areas bounded by the A and B curves on the resistivity tracing did not accurately represent the percentage of each component. The conductivities of pure kanamycin A and kanamycin B differed and did not remain constant with dilution within the concentration range of these experiments. Satisfactory quantitative analyses were carried out by using either gravimetric total solids or accurate refractive index measurements, since the latter did not appear subject to concentration differences. With these methods, the areas under the A and B curves were an accurate measurement of each component. For example, by plotting the individual cuts as in figure 2, the percentage of kanamycin B by the gravimetric method was 10.0 per cent and by refractive index, 9.4 per cent. A measurement of the areas under the resistivity curves, however, indicated 16.1 per cent kanamycin B. Each of these methods detected as little as 1 per cent kanamycin B.

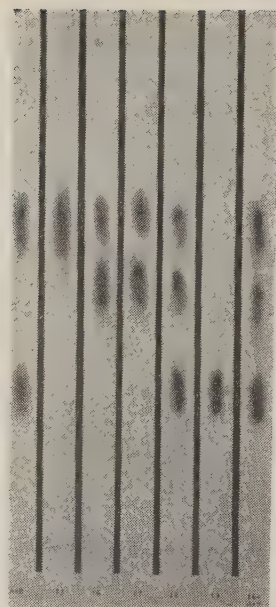
The resistivity tracing is similar in shape to the graph of total solids (figure 2). A plot of pH values also was of similar shape, but the range, only 0.4 unit, was too narrow to be useful. Because the biological assay is sensitive to both kanamycin A and B, the graph of the total biological activity is almost identical to the curves for the resistivity and total solids. Since the chemical assay is insensitive to the B com-

ponent, the plot of the total chemical activity is identical to the curves for biological activity, total solids, and resistivity only in the kanamycin A region. From the curves of total biological and chemical activity and total solids, it is evident that the kanamycin in each cut was not contaminated with inactive materials.



FIG. 4. The chromatographic separation of a crude kanamycin mixture on a Dowex 1-x2 resin column verified with bioautographs of paper chromatograms. Kanamycin A  $R_f$  0.13–0.18 and kanamycin B  $R_f$  0.26–0.28.

FIG. 5. Chromato red dyed paper chromatogram showing the composition of the eluate from a Dowex 1-x2 resin chromatography of a crude kanamycin preparation. Kanamycin A  $R_f$  0.13–0.18, kanamycin B  $R_f$  0.26–0.28, and kanamycin C  $R_f$  0.20–0.24. The channel paper strips read from left to right: A plus B, 13, 16–19, 16 plus A plus B.



The paper chromatograms (figs. 3 and 4) confirm the resin chromatographic separation of two components: kanamycin B at  $R_f$  0.26 to 0.28 and kanamycin A at  $R_f$  0.13 to 0.18. Kanamycin C was present in the eluate in too low a concentration to be detected. On the “chromato red” dyed strips (fig. 3), the feed solution was applied in the first channel, cuts 7 to 32 follow next in order, and a synthetic 1:1 mixture of A and B was applied in the last channel. No activity was detectable be-

FIG. 6. The composition of the eluate from a Dowex 1-x2 resin chromatography of a crude kanamycin preparation, depicted with a bioautograph of a paper chromatogram. Kanamycin A  $R_f$  0.12–0.18, kanamycin B  $R_f$  0.26–0.28 and kanamycin C  $R_f$  0.20–0.24.



fore cut 8. From the position on the resistivity tracing of cut 13, one would expect that it would be roughly an equal mixture of kanamycin A and B. Beyond cut 13, pure kanamycin A was obtained. There appears to be no contamination of kanamycin A in cut 12 or kanamycin B in cut 14. Similar results were observed with the more sensitive bioautographs (fig. 4).

The possibility of a third kanamycin component, which would give rise to the shoulder at cut 12 (fig. 1), was further investigated by chromatographing samples obtained earlier in the processing of fermentation broths. Crude IRC-50 eluate was chromatographed and the individual cuts assayed by the methods described earlier. A graph of total solids, total chemical activity, and total bioactivity was similar to the curve shown in figure 2. The paper chromatographic results are shown in figures 5 and 6. Both the dyed paper strips and the bioautograph plate show that a third component was isolated, which had a mobility approximately midway between kanamycin A and kanamycin B. Because the solvent front moved well beyond the end of the paper strip during the 40 to 48 hour development period, a direct measurement of  $R_f$  could not be made for the C spot. However, on the basis of  $R_f$  0.13 to 0.18 for A and 0.26 to 0.28 for B, the calculated  $R_f$  for kanamycin C was  $R_f$  0.20 to 0.24. The concentration of kanamycin C in the total eluted solids was estimated to be 2 to 3 per cent.

It should be noted that kanamycin C, although evident as a shoulder on all the resistivity tracings, was detected on paper chromatograms only in those samples obtained early in the isolation procedure. Apparently this component was largely lost in the preparation of crude, crystalline mono sulfate.

Umezawa and co-workers<sup>1</sup> reported a third component with antibacterial activity at  $R_f$  0 in the same developing system, but this substance was rarely produced. We have not detected this material.

#### SUMMARY

Crude crystalline kanamycin, isolated from fermentation broth by a resin process, was a mixture of several biologically active components. An analytical method has been developed to determine the amount of kanamycin A and kanamycin B in the mixture.

The crude kanamycin sulfate was decolorized and converted to the mixed bases with an anion-exchange resin. A sample was chromatographed on Dowex 1-x2 resin on the hydroxyl cycle, using water as a developing solvent, and the composition of the eluate was conveniently followed by means of a continual recording of the resistivity and by refractive index measurements of the individual cuts. The cuts were further analyzed for total solids, chemical activity, biological activity, and paper strip chromatograms were evaluated with dyes and by means of bioautograms to confirm the chromatographic separation and the reliability of the resistivity tracing. The area under the resistivity curves for kanamycin A and kanamycin B gave an erroneous value for the percentage of each component when compared with a gravimetric assay, whereas the area under the refractive index curve was more accurate.

A third biologically active component, designated kanamycin C, was observed as a shoulder in the resistivity tracing, and its presence was confirmed with dyed paper chromatograms and bioautographs. Kanamycin C was more mobile than kanamycin A and less mobile than kanamycin B in the *n*-butanol-water-*p*-toluene sulfonic acid system.

This chromatographic procedure was more rapid and more accurate than the conventional biological or paper chromatographic assay methods. As little as 1 per cent kanamycin B was determined. A sample was assayed easily within one working day.

#### ACKNOWLEDGMENT

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#### BIBLIOGRAPHY

1. UMEZAWA, H.; UEDA, M.; MAEDA, K.; YAGISHITA, K.; KONDO, S.; OKAMI, Y.; UTAHARA, R.; OSATO, Y.; NITTA, K., AND TAKEUCHI, T.: Production and isolation of a new antibiotic, kanamycin, *J. Antib.*, ser. A 10:181, 1957.
2. MAEDA, K.; UEDA, M.; YAGISHITA, K.; KAWAJI, S.; KONDO, S.; MURASE, M.; TAKEUCHI, T.; OKAMI, Y., AND UMEZAWA, H.: Studies on kanamycin, *J. Antib.*, ser. A 10:228, 1957.
3. CRON, M.; JOHNSON, D.; PALERMITI, F.; PERRON, Y.; TAYLOR, H.; WHITEHEAD, D., AND HOOPER, I.: Kanamycin. I. Characterization and acid hydrolysis studies, *J. Am. Chem. Soc.* 80:752, 1958.
4. SCHMITZ, H.; FARDIG, O.; O'HERRON, F.; ROUSCHE, M., AND HOOPER, I.: Kanamycin. III. Kanamycin B, *J. Am. Chem. Soc.* 80:2911, 1958.
5. PETERSON, D., AND REINECKE, L.: A paper chromatographic technique and its application to the study of new antibiotics, *J. Am. Chem. Soc.* 72:3598, 1950.
6. CONN, J., AND NORMAN, S.: Amperometric micro-titration of streptomycin and dihydrostreptomycin, *J. Clin. Invest.* 28:837-839, 1949.
7. STAPLEY, E.: Cross resistance studies and antibiotic identification, *Appl. Microbiol.* 6:392-398, 1958.

# Observations on the Treatment of Acute Gonococcal Urethritis in the Male with Intramuscular Kanamycin

## A Preliminary Report

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Kanamycin,\* a new antibiotic isolated from *Streptomyces kanamyceticus*, is active against a wide variety of gram-positive and gram-negative organisms.<sup>1</sup> Pilot studies at this hospital indicated that the intramuscular injection of kanamycin was well tolerated by patients and that pain at the site of injection was mild and did not exceed that experienced with penicillin. Because of this characteristic, the antibiotic was considered as a possible substitute for penicillin in the treatment of patients with gonorrhea, especially those who have a history of allergic sensitization to penicillin.

This paper, as a preliminary report, presents our results with intramuscular kanamycin in the treatment of 28 patients with acute gonococcal urethritis.

### METHODS

The patients in this study were unselected as regards allergic reactions to penicillin; all showed laboratory and clinical evidence of gonorrhea, and none had received any medication for the present infection. Details of the methods employed have been described previously.<sup>2</sup>

Kanamycin for intramuscular administration was available in vials containing 1 Gm. of the antibiotic dissolved in 3 ml. of distilled water. Treatment consisted of injections of kanamycin in the following dosage schedules: a single injection of 1 Gm., 8 patients; a single injection of 1 Gm. on two successive days, 10 patients; a single injection of 1 Gm. on three successive days, 10 patients.

### RESULTS AND DISCUSSION

Table I presents the results obtained under the different dosage schedules.

All patients receiving three injections of 1 Gm. each on three successive days responded favorably to treatment. The purulent discharge disappeared and the urethral burning sensation ceased. Two Gm. of the antibiotic gave less satisfactory results, and there were three failures among 10 patients. We could not ascertain, however, whether these represented relapses or reinfections, since all these patients admitted having sexual relations during the period of observation. Trials with this dosage, as well as other dosage levels, are continuing. One Gm. of kanamycin is insufficient for the treatment of gonorrhea.

We encountered no untoward reactions to kanamycin in any of our patients under these treatment schedules. None of the patients complained of ill effects.

Sensitization to penicillin is occurring with greater frequency, and with the passage

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This investigation was supported, in part, by a grant from Bristol Laboratories Inc., Syracuse, N. Y., and the kanamycin was furnished by them.

\* The trade name of Bristol Laboratories Inc. for kanamycin is Kantrex.

TABLE I  
*Kanamycin in the Treatment of Gonorrhea in the Male*

Total dose, Gm.	Dosage schedule	Patients treated and observed	Results	
			Cures	Failures
3	1 Gm. intramuscularly on 3 successive days	10	10	0
2	1 Gm. intramuscularly on 2 successive days	10	7	3
1	1 Gm. intramuscularly on 1 day	8	3	5

of time, more patients visiting the clinic give histories of allergic reactions to this drug. There is need for another injectable antibiotic for the mass treatment of venereal disease. Although our present series is small and further study is necessary, it appears from our preliminary observations that kanamycin may prove to be an effective intramuscular medication for the treatment of gonorrhea when the use of penicillin is contraindicated.

#### SUMMARY

Twenty-eight patients with gonococcal urethritis were treated under various dosage schedules. Three Gm. of kanamycin administered intramuscularly appear to be an effective dosage for the treatment of this disease.

#### BIBLIOGRAPHY

1. GOUREVITCH, A.; HUNT, G. A., AND LEIN, J.: Antibacterial activity of kanamycin, *Antib. & Chemo.* 8:149, 1958.
2. MARMELL, M., AND PRIGOT, A.: Intramuscular oxytetracycline in the treatment of acute gonorrheal urethritis in the male, *Internat. Rec. Med. & G.P.C.* 168:254, 1955.

# Kanamycin in Pediatric Infections

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The clinical evaluation of any new antimicrobial agent must include data related to the effectiveness of the agent, the toxicity of the agent, safe dosage schedules, and the conclusion as to whether or not the antimicrobial in question is better than already established agents for the treatment of infections. To satisfy inclusion in our present armamentarium of antimicrobials, any new drug must have a wide range of action with a low level of toxicity. With this background in mind, an attempt has been made to evaluate clinically the use of kanamycin in pediatric infections.

The methodology involved first a study of the effectiveness of kanamycin against *Salmonella* and *Shigella* infections; this experience has been reported previously.<sup>1</sup> Not only was an attempt made to define the effectiveness of this agent, but it was also necessary to establish safe dosage levels for children of all ages. The end result of this study must always be related to efficacy and toxicity. On conclusion of the original work, it was decided to test this agent against many other pediatric infections, for some of which we have, at present, adequate therapy, but also other infections that at the moment are unsatisfactorily treated with a wide variety of drugs. The next major groups treated were impetigo and other skin infections; some of these infections in our area had been remarkably resistant to both parenteral and local therapy. At the conclusion of this limited evaluation, it was then decided to use the drug in general medical problems of all types involving other organ systems. Throughout the study, dosage levels were calculated against the appearance of toxic effects in an attempt to determine maximal and minimal ranges associated with effectiveness and toxicity.

The bacteriological screen against which this agent has been tried is illustrated in table I. To our knowledge there has been no clinical experience at present, either here or in other areas, in the treatment of childhood tuberculosis. The results as summarized in table I indicate that kanamycin has much the same activity in childhood infections as it does in those of adults; the one difference noted here has been that this drug has had good activity against streptococcal infections. This has not been true in some of the previously reported experience in adults.<sup>2</sup>

## ROUTE OF ADMINISTRATION

In our experience, this antimicrobial has been used intravenously, intramuscularly, orally, and locally. It has also been reported previously<sup>3,4</sup> that this drug has been used by intraperitoneal and intrapleural instillation as well as in aerosol form. There has been no experience here with these forms of therapy. The clinical sample of intravenous administration is so small as to be insignificant.

The intramuscular administration of kanamycin has been noted to give local pain, erythema, and/or nodularity in approximately 12 per cent of the patients treated. These effects at no time have necessitated discontinuation of the drug. Several times it was noted that the drug was given improperly in a subcutaneous

TABLE I  
*Response to Therapy Classified by Organism*

Organism	Cases	Satisfactory	Unsatisfactory	Indeterminate
<i>Aerobacter aerogenes</i>	2	2	—	—
<i>E. coli</i>	4	2	1	1
<i>Klebsiella</i>	5	3	2	—
<i>Mimae</i>	3	2	—	1
<i>Neisseria gonorrhoeae</i>	2	1	1	—
<i>Paracolon</i>	2	2	—	—
<i>Pneumococcus</i>	8	6	1	1
<i>Proteus</i>	8	4	2	2
<i>Pseudomonas</i>	9	2	5	2
<i>Salmonella</i>	19	11	5	3
<i>Shigella</i>	57	44	8	5
<i>Staphylococcus</i>	13	10	1	2
<i>Streptococcus</i>	12	9	2	1
Total	144	98	28	18

fashion, but this has not caused local destruction of tissue or formation of sterile or unsterile abscesses.

Oral administration of the drug has been carried out both in capsule form and in the new liquid suspension form. There has been no significant difference in side effects between the capsule or the liquid suspension, and both have proved efficacious in the treatment of gastrointestinal infections; as is commonly true in the treatment of children, the liquid form has proved to be much more acceptable than the large capsules. There have been no complaints of unpleasant aftertaste or of a granular sensation within the oral cavity, as has been reported with other liquid suspensions.

Local applications of the agent have been administered in a lanolin base, and there have been no allergic manifestations or other evidences of toxicity in this type of therapy. Our conclusions are summarized in table II; regardless of the route of administration of this agent, it has a low incidence of major and minor side effects.

#### DOSAGE LEVELS AND TREATMENT INTERVALS

Original testing was carried out with dosage levels of 15 mg./Kg. in infants and children of all ages. Bacteriological studies and clinical evaluation established

TABLE II  
*Incidence of Toxicity Related to Route of Administration*

Toxic reaction	Cases	Intravenous	Intramuscular	Oral	Local
Cranial nerve involvement	—	—	—	—	—
Dizziness	—	—	—	—	—
Eosinophilia	5	1	3	—	1
Headache	—	—	—	—	—
Local pain	17	—	17	—	—
Skin rashes	1	—	1	—	—
Urine changes	37	2	31	4	—
Yeast overgrowth	1	1	1	1	—
Total	61	4	53	5	1

TABLE III  
*Toxic Reactions Related to Dosage*

Type of reaction	Cases	mg./Kg.		
		15	25	50 or more
Cranial nerve involvement	—	—	—	—
Dizziness	—	—	—	—
Eosinophilia	5	3	1	1
Headache	—	—	—	—
Local pain	17	5	7	5
Skin rashes	1	1	—	—
Urine changes	37	9	13	15
Yeast overgrowth	1	—	—	1
Total	61	18	21	22

that this dosage was not adequate to manage most acute infections. Trials were then continued with the dosage level increased to 25 mg./Kg., and it was found that 80 per cent of the infections so treated responded well, both clinically and bacteriologically. To attempt to establish maximal dosage related to minimal toxicity, other cases were treated with 50 mg./Kg., and in several septicemias, dosage levels as high as 100 mg./Kg. have been tried. It has been consistently true that the higher the dosage, the more frequent the appearance of toxic changes in the urinary sediment, usually starting about the third day of the elevated dosage. However, even at the elevated dosage level, urinary changes have been found to be reversible once the drug has been stopped. It has been the consensus of opinion that 25 mg./Kg. of this agent is satisfactory for the control of most pediatric infections. We have not hesitated to go to higher levels in the face of severe infections, and our decisions have been justified both by response and by minimal toxicity; these experiences are summarized in table III.

Considering the possibility of eighth nerve involvement associated with prolonged therapy, we have avoided continuous use of this antibiotic. Our longest interval of therapy to date has been 18 days, with the average being five to seven days and a minimum of three days of complete therapy. The results of this interval schedule of dosage related to toxicity are summarized in table IV; as illustrated, the five to seven day interval of therapy has proved more efficacious with less toxicity than any other course of management. It has also been shown that five to seven days of therapy is usually adequate to control the infectious process.

TABLE IV  
*Duration of Therapy Related to Toxic Reactions*

Type of reaction	Cases	Interval of therapy			
		3 days	5-7 days	10-14 days	More than 14 days
Cranial nerve involvement	—	—	—	—	—
Dizziness	—	—	—	—	—
Eosinophilia	5	1	2	2	—
Headache	—	—	—	—	—
Local pain	17	5	5	6	1
Skin rashes	1	1	—	—	—
Urine changes	37	2	14	20	1
Yeast overgrowth	1	—	—	—	1
Total	61	9	21	28	3

## TOXICITY

The danger of any new antimicrobial is that it may have some suspected or unsuspected toxicity that will lead to more physiological damage than is caused by the original infectious process. In the 144 cases listed in this paper, only 21 patients have manifested any evidence of toxicity whatsoever; of those manifesting any toxic reaction, 74 per cent have shown two or more clinical evidences of toxicity. The possible toxic effects are listed in table III along with the incidence related to treatment with this drug. By far the most frequent have been changes in urinary sediment and albuminuria, but as previously stated, these changes are reversible after discontinuation of therapy. Most of the other toxic manifestations have been rare in our experience. In the younger infant it is extremely difficult to evaluate cranial nerve damage, particularly when it is necessary to make audiometric evaluations as well as tests of vestibular function to document the toxicity; however, many of our patients have been old enough to respond satisfactorily to questions related to these disturbances, and in this experience there has been no evidence of cranial nerve involvement whatsoever. Tables II, III, and IV summarize the incidence of toxic reactions associated with variables of therapy.

## PRECAUTIONS

Two findings of this clinical survey are worthy of mention under this heading.

First is the increased incidence as well as the more rapid onset of toxicity noted when kanamycin is given in large dosages to clinically dehydrated children. Whereas toxicity evidenced by urinary changes seldom appears prior to the third or fourth day of therapy, it has been noted to appear as early as the second day in children treated after episodes of nausea and vomiting with subsequent dehydration. This finding has been noted in 4 of the cases presented previously.

Subsequent to this experience, the therapeutic regimen in dehydrated children has been to start therapy with dosage levels of 15 mg./Kg. and gradually increase the dosage to either 25 or 50 mg./Kg. as clinically indicated. With this plan of management, toxicity is minimized.

The second precaution has been that the interval between doses should be no less than every eight hours, and preferably it should be every six hours. Several of the cases described were treated with injections at 12 hour intervals. It was noted that these patients did not respond clinically as well as did patients on more frequent injections. To test this supposition, two urinary infections, with the same etiological organism and essentially the same clinical picture, were treated with

TABLE V  
*Response to Therapy Related to Type of Infection*

Type of infection	Cases	Satisfactory	Unsatisfactory	Indeterminate
Cellulitis	6	5	1	—
Diarrhea	76	61	13	2
Furunculosis	4	2	1	1
Impetigo	18	15	2	1
Otitis media	10	6	3	1
Pneumonia	6	4	2	—
Septicemia	5	2	2	1
Typhoid fever	3	—	2	1
Urinary tract infections	8	6	1	1
Carrier stages	4	3	1	—
Others	4	2	2	—
Total	144	106	30	8

different schedules; the first patient, treated at six hour intervals, had a clinical and temperature response approximately 24 hours before these changes were noted in the second, treated at 12 hour intervals. Recent data<sup>5</sup> concerning serum levels have added laboratory evidence to clinical impression.

#### CONCLUSIONS

Table IV illustrates the type of infection treated with this antimicrobial. It indicates that kanamycin does fulfill one of the two criteria listed as necessary for inclusion as a worthwhile drug in our present armamentarium—that of a wide range of activity.

Preceding data have been presented to illustrate that it also fulfills the second criteria—low level of toxicity.

This experience also indicates that five to seven day courses of therapy at a dosage level of 25 to 50 mg./Kg. are efficacious in most pediatric infections with an associated low level of toxicity.

#### BIBLIOGRAPHY

1. THURMAN, W. G., AND PLATOU, R. V.: Some experiences with kanamycin in the treatment of *Salmonella* and *Shigella* infections, Ann. New York Acad. Sc. 76:230, 1958.
2. GOUREVITCH, A.; ROSSOMANO, V. Z.; PUGLISI, T. A.; TYNDA, J. M., AND LEIN, J.: Microbiological studies with kanamycin, Ann. New York Acad. Sc. 76:31, 1958.
3. HIGH, R. H.; SARRIA, A., AND HUANG, N. N.: Kanamycin in the treatment of infections in infants and children, Ann. New York Acad. Sc. 76:289, 1958.
4. PRIGOT, A.; SHIDLOVSKY, B. A., AND CAMPBELL, E. A.: Intraperitoneal use of kanamycin as an adjunct in the therapy of established peritonitis and peritoneal contamination, Ann. New York Acad. Sc. 76:204, 1958.
5. BERGER, S. H., AND WEHRLE, P. F.: Kanamycin serum levels in infants and children, Ann. New York Acad. Sc. 76:136, 1958.

# The Clinical Use of Kanamycin in Staphylococcal Infections

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Three types of staphylococcal disease problems were available for study with kanamycin. These included treatment of residents of an old-age home who had hypertensive-arteriosclerotic cardiovascular-renal disease and diabetes mellitus. These patients were frequent victims of staphylococcal infections, particularly of the skin. The second group of patients were those seen in private practice who were treated on an ambulatory basis. Actually the third aspect of this study consisted of evaluating the results in patients in the aged and private practice groups who had been treated unsuccessfully with other antibiotics prior to the use of kanamycin.

In all instances the causative organism was identified by cultural characteristics. Sensitivity studies were not performed.

There were 10 patients treated at the old-age home. The youngest patient was 69 and the oldest 82. All but 1 of the patients had diabetes mellitus and all had hypertensive-arteriosclerotic cardiovascular-renal disease. In all the patients there was a staphylococcal skin infection, such as pyoderma, folliculitis, or furunculosis. The staphylococcal infection complicated a herpes zoster infection in one instance. The kanamycin was given in dosages of 0.5 Gm. every 12 hours intramuscularly for five days in 6 patients, with prompt and uneventful recovery. In 2 patients treatment had to be continued for 10 days. Of this group, 2 additional patients recovered, for a total of 8 out of 10. In 2 patients treatment was discontinued at the end of this time without any significant improvement.

Fifteen patients with a variety of staphylococcal infections have been treated with kanamycin on an ambulatory basis as part of a private practice. There were 3 patients with urinary tract infections, 1 with prostatitis, 2 with secondary infected herpes zoster, 2 with otitis media, and 7 with skin infections, such as pyoderma, folliculitis, furunculosis, impetigo, and carbuncles.

All patients received 1 Gm. of kanamycin in a single intramuscular injection daily for seven days, except the patient with prostatitis, who was treated for 10 days. Treatment was successful in all except 2 patients—one with otitis media and another with furunculosis. There was no evidence of improvement after seven days of therapy in these patients, and the kanamycin was discontinued.

The third aspect of this study was to evaluate the usefulness of kanamycin in staphylococcal infections resistant clinically to other antibiotics. Twelve patients, 5 from the old-age home group and 7 from the private practice group, had been treated with other antibiotics—penicillin, streptomycin, erythromycin, tetracycline-nystatin, oxytetracycline, chloramphenicol, and tetracycline, alone or in various combinations. Four of the 5 in the resistant group from the old-age home responded to kanamycin. Six of the 7 from the private practice group found resistant to other antibiotics responded to kanamycin.

There was no evidence of any adverse effect on the blood cells except a reduction in the number of leukocytes and polymorphonuclear leukocytes as the infection improved. There were no changes in the urine except in the presence of prostatitis and urinary tract infections, where the abnormal findings disappeared

with treatment. The blood and urine were examined at the start and conclusion of therapy in each patient. There were no evidences of any abnormal findings or symptoms clinically.

It is apparent that kanamycin is a useful drug in the treatment of staphylococcal infections, particularly those resistant to other antibiotics. It can be used for periods of time up to 10 days without any toxic or side effects even in elderly patients with hypertension, arteriosclerosis, impaired circulatory, cardiac, and renal function, and in the presence of diabetes mellitus. Even though kanamycin is useless in systemic infections when given by mouth, effective treatment can be accomplished by daily intramuscular injections of 1 Gm. daily. Such a simple program can easily be employed in private practice.

# An Evaluation of Different Types of Paper Concerning Their Suitability for Sensitivity Discs

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The use of the dry antibiotic disc in bacterial sensitivity testing has received wide acceptance in hospital laboratories. This is in spite of the many papers published criticizing the poor standardization of such discs.<sup>1,2</sup> One common criticism is directed at the poor control of the paper by the commercial manufacturers of the discs. An often repeated statement is that discs made from different papers with different *pH* values, thicknesses, and diameters give quite different releases.<sup>3</sup> Since experimental evidence of this is difficult to find in the literature, a series of experiments were set up as a preliminary to confirming or refuting this statement. Our interest in the problem is an outgrowth of staphylococcal research which includes sensitivity testing.

Sixteen papers obtained from various sources were tested for their releasing power to penicillin. Also tested were the influence of *pH* of the papers, the thickness and diameter of the disc, and volume of liquid used to impregnate the disc with two units of penicillin.

## MATERIALS AND METHODS

The discs were tested according to the Pure Food and Drug Administration method. Forty-two ml. of base agar were poured into 150 mm. Petri dishes. This was overlaid with 8 ml. of agar inoculated with a sensitive strain of *Micrococcus pyogenes* var. *aureus* (ATCC 6538P). After the discs were added, the plates were incubated at 37 C. for 18 hours and the zones of inhibition measured.

Sixteen different papers were cut into 6.35 mm. discs and sterilized in the hot air oven for one hour at 125 C. They were impregnated with 2 units of penicillin\* contained in 0.01 ml. of water and dried at 37 C. The papers tested were Schleicher and Schuell discs 740E; two filter papers; a Spinco filter paper electrophoresis wick; eight blotter papers, six of which contained different dyes; and four papers used in commercial sensitivity discs. Adsorption experiments were performed on some of these papers by adding 3.5 ml. of water containing 2 units of penicillin per ml. to 0.5 Gm. of macerated paper in a test tube. These tubes were incubated at 37 C. for 18 hours and the supernatant tested for penicillin content by the cylinder plate method.

The *pH* of the papers was obtained by macerating the paper dampened with neutral distilled water and measuring with a *pH* meter.

The effect of different *pH* values of the paper on the releasing power of the disc was determined by varying the *pH* of a single paper (Spinco wick) from 6.0 to 8.0 with 1/15 *M* phosphate buffer. Discs cut from this paper were treated and tested as just indicated.

To test the effect of paper thickness, Schleicher and Schuell discs were employed. Some of the discs were sliced in half and impregnated with 2 units of penicillin and others were treated in their full thickness.

\* Assay standard furnished by the Chas. Pfizer & Co.

Cork borers were used to cut one type of paper (Spinco wick) into discs of various sizes. Each size disc was given 2 units of penicillin and the zones of inhibition compared.

Various concentrations of penicillin were made up to test the effect of volume of solution added to the size of the zone of inhibition. Discs measuring 12.5 mm. were treated with 2 units of penicillin contained in 0.005, 0.01, 0.02, and 0.04 ml. of water.

#### RESULTS AND DISCUSSION

The zones of inhibition of the 16 papers varied only slightly, as is shown in figure 1. The only marked variation was in three of the dyed discs. The nine undyed discs differed by only 2.5 mm. from the smallest (31 mm.) to the largest (33.5 mm.) zones. Since this includes qualities of paper ranging from the common desk blotter to high grade filter paper, it would seem that most good quality papers would be acceptable. The question does arise here, however, as to whether dyes

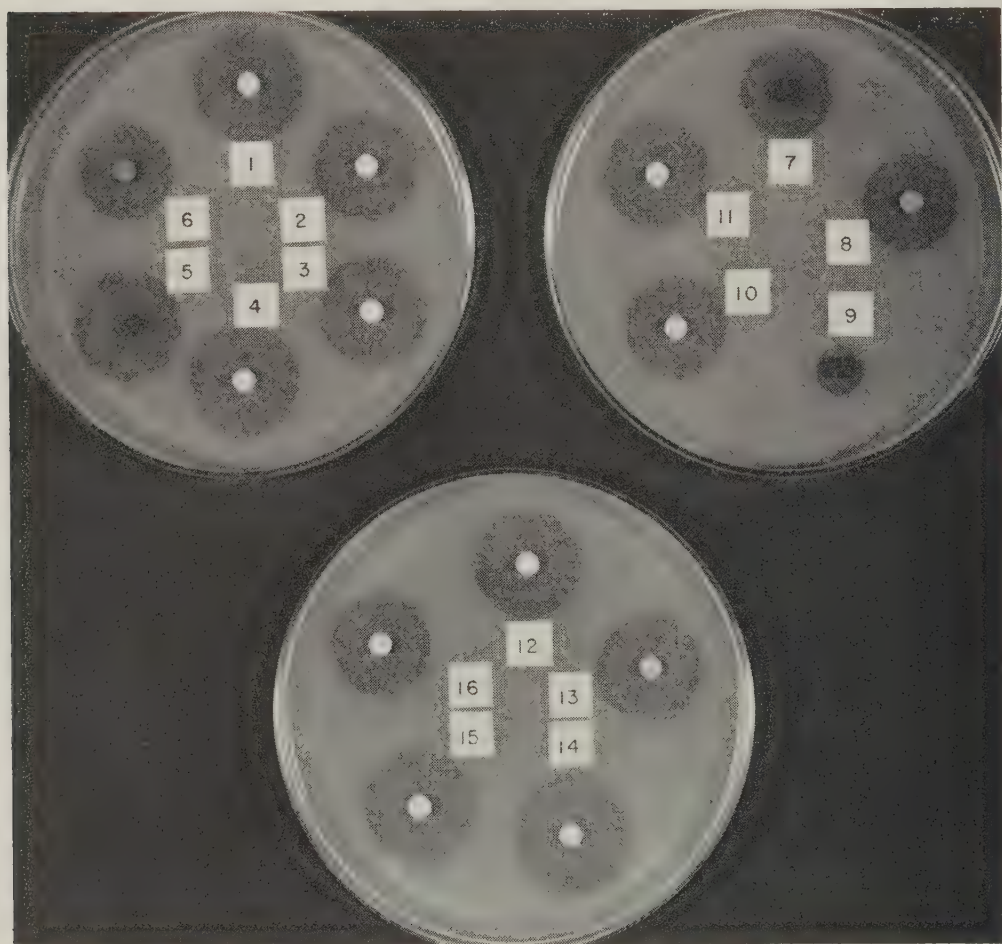


FIG. 1. Zones of inhibition produced by 16 papers. Diameter of zones varied from 14.0 mm. for paper 9 to 33.5 mm. for paper 1. The papers consisted of: 1, 185 mm. filter paper; 2, blotter paper; 3, Spinco filter paper wick; 4, Whatman no. 42 filter paper; 5 to 10, dyed blotter papers; 11, Canadian blotter paper; 12, Schleicher & Schuell discs no. 740E; 13, green fordiscs blanks for chloramphenicol; 14 to 16, paper used in manufacture of penicillin discs.

FIG. 2 Zones produced by cylinder-plate method on supernatant after adsorption of penicillin solution by paper. Two units of penicillin per ml. used in experiment; 0.1 ml. added to cylinder. C is the control.

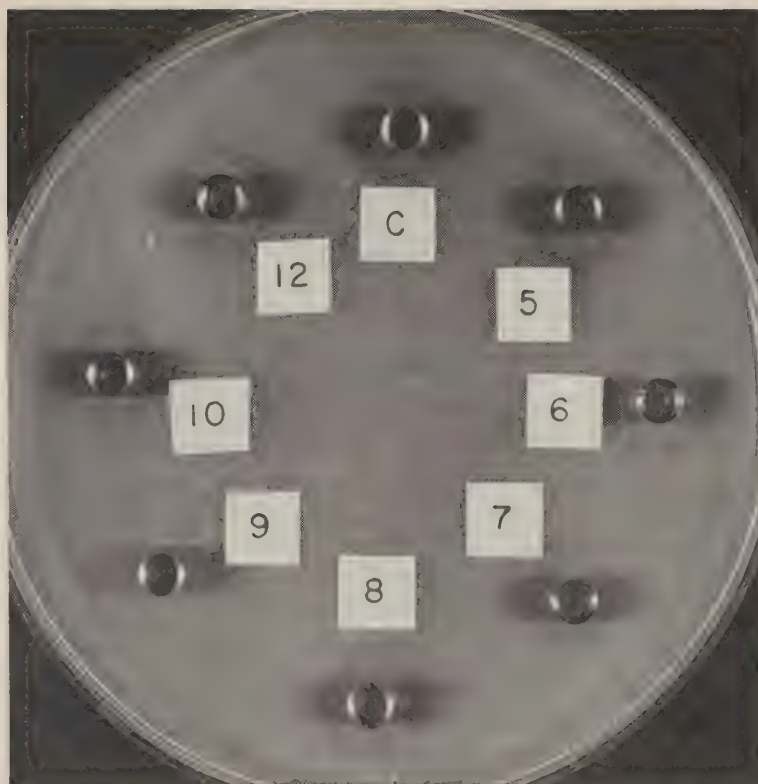


FIG. 3. Effect of paper thickness. These discs are of the same paper, one series twice as thick as the other. Two units of penicillin added to both.



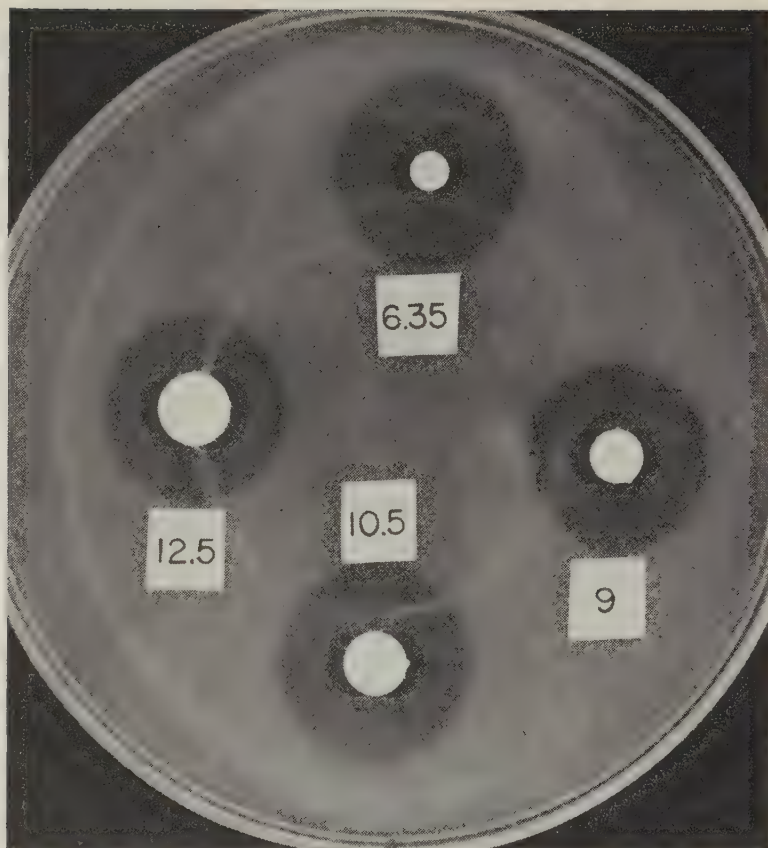


FIG. 4. Effect of paper diameter. All discs are from the same paper and each was impregnated with 2 units of penicillin. The smallest zone is 31.5 mm. and the largest is 32.5 mm.

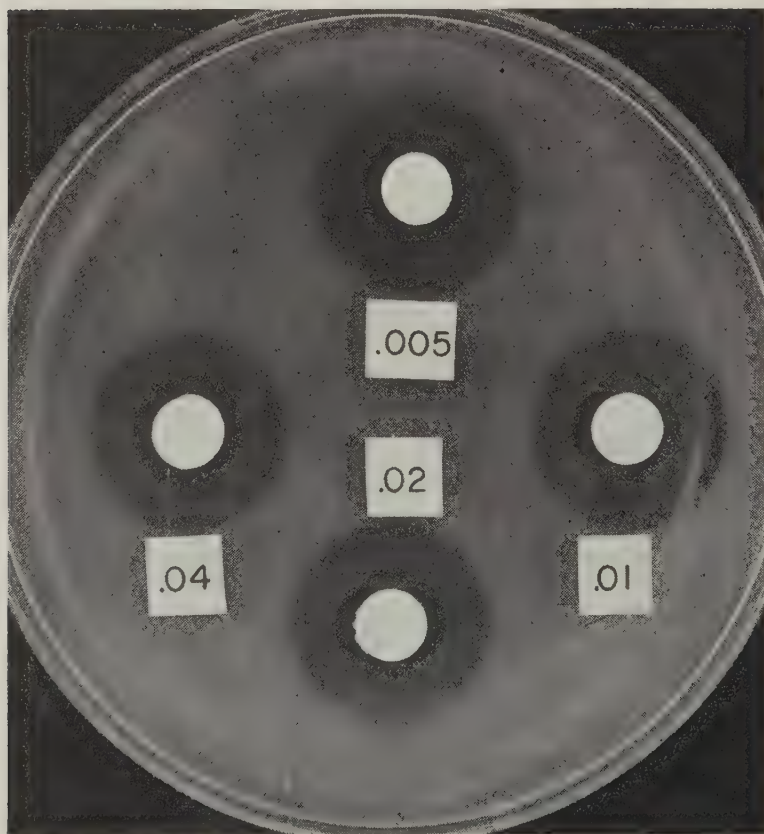


FIG. 5. Effect of volume added to 12.5 mm. discs. Volumes added ranged from 0.005 ml. to 0.04 ml. Each amount contained 2 units of penicillin. Greatest deviation in size was 1 mm.

should be used. Four of the seven dyed discs failed to inhibit growth after they were stored in the refrigerator for eight days without a desiccant.

The plain paper itself does not seem to inactivate or bind the penicillin as was shown by the adsorption experiment. One of the dyed papers did inactivate or adsorb the penicillin, since drug activity could not be demonstrated in the supernatant. This is shown in figure 2.

The pH values of these papers ranged from 6.3 to 7.6. In testing the effect of paper pH on the release of penicillin, it was found that pH between 6.0 and 8.0 had little or no effect on the zones of inhibition. Since all of the papers tested fell within this range, it appears that the pH is of little importance. Since, however, these discs were tested within 24 hours after preparation, the effect of pH on storage is not known.

The thickness of the paper does not appear to be of much consequence as is shown in figure 3. As long as they contain the same amount of penicillin, the zones seem to be equal.

The same is true in regard to the diameter of the disc as is noted in figure 4. The sizes tested ranged from 6.35 to 12.5 mm. The diameter of the zones did not vary by more than 1 mm.

Some question has been raised as to whether the antibiotic should be equally distributed throughout the disc. In an attempt to find the answer to this question, solutions of penicillin in various concentrations were placed on the 12.5 mm. discs. The volumes used ranged from 0.005 ml. to 0.04 ml.; each volume containing 2 units of penicillin. Figure 5 shows that this did not effect the size of the zone of inhibition.

Although this is just a preliminary report dealing only with penicillin and one organism, the results thus far obtained suggest that the paper does not play so important a role as thought. If dyes are used, however, they should be carefully checked for any action they may have on the antibiotic. The main problem in the standardization of sensitivity discs appears to be in securing the proper amount of antibiotic in the disc.

#### SUMMARY

Sixteen papers were tested for their releasing power of penicillin. Only the dyed papers gave a marked reduction of the zone of inhibition. All of the papers fell within a pH range that does not seem to affect the release of penicillin from the disc. The thickness and diameter of the disc did not affect the zone of inhibition. Likewise, the volume used to impregnate the disc was shown to be of no concern.

#### BIBLIOGRAPHY

1. GREENBERG, L.; FITZPATRICK, K. M., AND BRANCH, A.: The status of the antibiotic disc in Canada, *Canad. M. A. J.* 79:194-195, 1957.
2. HOFFMAN, R. V., JR.; JACKSON, G. G., AND TURNER, M. P.: Reliability of antibiotic sensitivity tests as determined by a survey study, *J. Lab. & Clin. Med.* 51:873-882, June 1958.
3. KIRSHBAUM, A.; KRAMER, J., AND ARRET, B.: The assay and control of antibiotic sensitivity discs. Unpublished.

# Antibiotic Concentrations in Discs Used for Susceptibility Testing of Staphylococci

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Previous experience with topical antibiotic therapy and in vitro susceptibility testing<sup>1,2</sup> suggested that lower concentrations of antibiotics in discs might yield minimum inhibitory values that correlated better with tube dilution results and clinical response. Also, there is some controversy concerning the proper concentrations in antibiotic discs, and in recent years the concentrations in commercially prepared discs were changed.

The purpose of this paper is to report the distribution of minimum inhibitory concentrations (MIC) among susceptible and resistant strains of *Staphylococcus aureus* tested by both broth dilution and disc (agar diffusion) techniques with eight antibiotics.

## MATERIALS AND METHODS

Special discs were obtained having lower concentrations of antibiotics than those available commercially. The antibiotics tested were bacitracin, erythromycin, neomycin, novobiocin, tetracycline, penicillin G, chloramphenicol, and oxytetracycline. The concentrations used for the discs and tubes are shown in tables I and II. Only single discs were used; none was connected with another in "rings" or "stars." The techniques used were those reported previously.<sup>3</sup> The staphylococci tested were isolated during a three year period (1955 to 1958) from both outpatients and inpatients observed in a study of infected dermatoses.

Because the concentrations in the antibiotic discs were changed from time to time, and all materials were not simultaneously available, the following system of recording the inhibition of organisms by discs was devised by one of us (J. E. G.). The results were recorded as an improper fraction, the numerator of which was the MIC in that test, and the denominator was the lowest concentration of a particular antibiotic used in that test. Thus, the result 0.5/0.5 for erythromycin meant that the organism was inhibited by a disc containing 0.5  $\mu$ g. of erythromycin, which was the smallest amount of erythromycin used in that trial. The result 10/2 meant that the organism was inhibited by a 10  $\mu$ g. disc but not by a 5 or a 2  $\mu$ g. disc. Results in which the organisms were resistant to the highest concentration employed were recorded with an R before the number of  $\mu$ g. in the highest concentration disc used for that test. These results were finally grouped into the three classifications of susceptible (S), moderately resistant (M), and resistant (R), as indicated in table I. In some instances all available discs were used initially to test the organisms. More often, however, only the lowest concentration disc was used, the remaining higher concentrations being employed only if the organisms were resistant to the lowest concentration.

## RESULTS

The MIC of the eight antibiotics in multiple tube dilution tests for *Staph. aureus*

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This investigation was aided by a grant from The Upjohn Company, Kalamazoo, Mich.

TABLE I  
Criteria for Classification of Susceptibility Results  
and Concentrations of Antibiotic Discs Used

Antibiotics*	Values of inhibiting discs		
	Susceptible	Moderately resistant	Resistant
Bacitracin	<1,† 1, 2	10	20
Chloramphenicol	1, 5	10, 30	60
Erythromycin	0.5, 1, 2	5, 10, 15	
Neomycin	1, 2, 5	10, 30	60
Novobiocin	5	30	100
Penicillin	0.5, 1, 2	5, 10	
Oxytetracycline	1, 5	10, 30	60
Tetracycline	1, 2, 5	10, 30	60

\* Concentrations of all antibiotics are in  $\mu\text{g./disc}$  except for bacitracin and penicillin, which are in units.

† These discs were those used for differentiating group A streptococci from other  $\beta$ -hemolytic streptococci.

are shown in table II. In no case is there an even distribution of organisms. Rather, they are distributed in clusters as either susceptible or resistant. With bacitracin, there is little variation, for staphylococci are very rarely resistant to this antibiotic. With chloramphenicol, there is little variation with this sample. With erythromycin, penicillin, oxytetracycline, and tetracycline, there are marked differences between susceptible and resistant organisms. With neomycin, there is little variation because these are coagulase-positive staphylococci. All were resistant to  $0.25 \mu\text{g./ml.}$ , and most were resistant to  $1 \mu\text{g./ml.}$  With novobiocin, most strains were susceptible; only recently have the few moderately resistant strains been observed.

Although data for only coagulase-positive staphylococci (*Staph. aureus*) are shown here, results with coagulase-negative staphylococci (*Staph. epidermidis*) have been similar in this laboratory and elsewhere<sup>4</sup> by both disc and multiple tube dilution techniques.

Results of the disc tests are shown in table III. Staphylococci inhibited by any of the discs listed in the "susceptible" column of table I were classed as susceptible. Although zone sizes varied according to the concentrations employed, no useful distinction between these low concentration discs has been observed. Staphylococci inhibited by any one disc of this set were also inhibited by the others of a given antibiotic, except for neomycin (*vide infra*). Those classed as moderately resistant

TABLE II  
Inhibitory Concentrations of 8 Antibiotics in Broth for  
*Staph. aureus*

	Total no. staphylococci tested	MIC, $\mu\text{g./ml.}$ or units/ml.								
		0.1	0.25	0.5	1.0	2.5	5	10	25	R25*
Bacitracin	125			2		38	58	27		
Chloramphenicol	60						29	29	2	
Erythromycin	60		1	45	13					1
Neomycin	125			2	20	69	33	1		
Novobiocin	139	32	76	18	2	1	1	9		
Penicillin	60	30			1	1	4	3	5	16
Oxytetracycline	60		16	28	2				1	13
Tetracycline	60		31	6	9					14

\* The numbers represent those staphylococci resistant to 25 units or  $\mu\text{g./ml.}$

TABLE III  
*Antibiotic Disc Susceptibility of*  
*Staph. aureus*

	Total	Sensitive	Moderately resistant	Resistant
Bacitracin	924	923	1	0
Chloramphenicol	284	261	20	3
Erythromycin	2355	2241	5	109
Neomycin	2355	2352	3	0
Novobiocin	2161	2135	26	0
Penicillin	2278	988	166	1124
Oxytetracycline	230	150	0	80
Tetracycline	2357	1660	87	610

(table III) were inhibited by discs listed in that column in table I. Resistant organisms were those not inhibited by any discs in the center column or only those in the "resistant" column of table I. Organisms with zones less than 9 mm. in diameter around the upper levels of the medium concentration discs were classed as resistant.

With bacitracin, only one *Staphylococcus* of 924 was observed that gave the result 10/2; none was resistant to the 20 unit disc.

With chloramphenicol, most organisms were inhibited by the 1 and 5  $\mu$ g. discs; 20 were recorded as 30/5, and 3 as R30.

With penicillin, there were more resistant staphylococci than with any other antibiotic tested. A majority of the 166 moderately resistant organisms was detected in the first two years, which is attributed to the production of more uniformly potent discs. That the "M" classification with penicillin is a definite entity is shown by the results with organisms from cultures repeated weeks or even months later on the same patients. The organisms were numbered and tested independently without knowledge of the previous results, yet consistently gave the "M" type reaction.

Differences in low concentration discs were not distinguishable except by measurement of zone sizes. The 2 unit disc appeared to be the most satisfactory low concentration disc for penicillin.

With erythromycin, staphylococci inhibited by the 2  $\mu$ g. discs were inhibited by the 1 and 0.5 as well. Only five staphylococci were not inhibited by these but were by the 10 or 15  $\mu$ g. discs. None of 2355 was inhibited by 5 but not by 2  $\mu$ g. or less.

Neomycin discs of 1  $\mu$ g. inhibit *Staph. epidermidis* but rarely *Staph. aureus*.<sup>3</sup> Of 2355 staphylococci tested, 2 or 5  $\mu$ g. discs inhibited all but three, which were inhibited by either 10 or 30  $\mu$ g. discs.

With novobiocin, 2135 strains gave results recorded as 5/5; 26, as 30/5; none, as R30 or R100.

With oxytetracycline and tetracycline, disc susceptibility tests showed a high correlation (99.3 per cent) with each other. Multiple tube dilution tests agreed. Because of this cross resistance, routine tests with oxytetracycline were discontinued. Some of these tests were performed with the tetracycline phosphate complex rather than the hydrochloride, but no difference was observed in the results with either radical, the activity being similar.<sup>5</sup> As with penicillin, the number of moderately resistant organisms decreased in the latter part of the study.

#### DISCUSSION

These data do not reflect the true incidence of resistant staphylococci in patients

seen in this department, since this sample did not include all patients, and many organisms were from multiple cultures from the same patient.

It has been previously shown that, in general, the antibiotic susceptibility, by broth dilution, of bacteria isolated from cutaneous pyogenic infections parallels clinical response.<sup>1,2</sup> It is well established that broth dilution and disc susceptibility tests are well correlated. This was also true in this study. Much of the criticism of the disc technique is the result of inaccuracies due to (1) improperly performed tests, (2) misinterpretation of results, and (3) inaccurately manufactured discs. Discussions of these first two may be found in most articles on bacterial susceptibility testing. The third also has been the topic of several papers<sup>6-8</sup> and the manufacture of discs has now come under closer government control in both Canada and the United States.

Susceptibility tests with bacitracin have been difficult because this antibiotic is not only thermolabile but also has only a biological assay and not a chemical assay. Penicillin is also a difficult antibiotic to use in dry discs because of its thermolability.<sup>6</sup> Anomalous results and many inaccuracies with both neomycin<sup>3</sup> and streptomycin discs have been observed in this laboratory and elsewhere. This has resulted from the use, by some manufacturers, of ethylene oxide for the sterilization of discs. Ethylene oxide rapidly destroys the activity of neomycin and streptomycin.<sup>9</sup>

In this laboratory, the single type disc in vial, or cartridge for dispenser, has been the most satisfactory, combining convenience with flexibility. Combined discs, i.e., those in which several antibiotics are joined together in "rings" or "stars" are convenient but undesirable since an error in one antibiotic necessitates discarding the entire group or cutting out a segment. Both laboratory personnel and manufacturers are reluctant to do either.

The data of tables II and III show the tendency for staphylococci to be either highly susceptible or highly resistant, with few intermediates, to the antibiotics tested. Therefore, the results may be ascertained with only two discs. Indeed, one disc is sufficient if the potency and the zone size range are standardized. This has been recommended by Kirby et al<sup>10</sup> and found satisfactory in this laboratory. They reported few results in the "middle zone" with oxytetracycline, chloramphenicol, streptomycin, polymyxin B, penicillin, erythromycin, bacitracin, and sulfisoxazole. Also they found generally good correlation between results using single discs and three discs of different concentrations. In few instances, three discs were better than one when one gave borderline results. For this reason, the two disc method has proved best in this laboratory, since detecting the presence or absence of a zone is easier and quicker than measuring zone sizes, and the use of discs in dispensers eliminates the extra labor involved in placing an additional disc.

The data of Engley and Bass<sup>11</sup> and Vaccaro et al<sup>12</sup> are also compatible with the results and conclusions presented here, indicating their wider application than just to staphylococci from patients with cutaneous bacterial infections.

#### SUMMARY

The distribution of minimum inhibitory concentrations among susceptible and resistant strains of *Staphylococcus aureus* tested by both broth dilution and disc (agar diffusion) techniques with eight antibiotics is reported. These data show these values to be mostly at either extreme, susceptible or resistant. Thus, two discs are sufficient to classify staphylococci as susceptible, moderately resistant, or resistant, since few intermediates occur. One disc is sufficient if concentrations and zone sizes are properly standardized. Some reasons for inaccurate discs are discussed.

The discs used in this study were manufactured and generously supplied by the Baltimore Biological Laboratories, Baltimore, Md., and the Difco Laboratories, Detroit, Mich.

The bacitracin, erythromycin, neomycin, novobiocin, tetracycline, and penicillin used in this study were kindly supplied by The Upjohn Co.; the chloramphenicol by Parke, Davis & Co., Detroit, Mich.; and the oxytetracycline by Chas. Pfizer & Co., Brooklyn, N. Y.

## BIBLIOGRAPHY

1. LIVINGOOD, C. S.; HEAD, E. S.; JOHNSON, E. A., AND NILASENA, S.: Erythromycin in local treatment of cutaneous bacterial infections, *J.A.M.A.* 153:1266, 1953.
2. LIVINGOOD, C. S.; HEAD, C. E.; SUTTER, R. M., AND ENGLE, F. B., JR.: Comparative effects of neomycin and other antibiotics on pyogenic bacteria. In vitro effect on bacteria isolated from cutaneous pyogenic infections, *A.M.A. Arch. Dermat. & Syph.* 69:43, 1954.
3. GREER, J. E., AND MENARD, R. R.: Neomycin susceptibility for differentiating coagulase-positive from coagulase-negative staphylococci. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 149-153.
4. GRIFFITH, R. S.; BONIECE, W. S.; MCGUIRE, J. M.; WOLFE, R. N.; JOINER, M.; WICK, W. E., AND HOLMES, D. H.: Biological and antibiotic sensitivity studies on "Street Staphylococci." *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 370-379.
5. CRONK, G. A.; NAUMANN, D. E., AND CASSON, K.: Clinical and laboratory experience with tetracycline phosphate complex. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 397-408.
6. BRANCH, A.; STARKEY, D. H.; POWER, E. E., AND GREENBERG, L.: Problems of standardization of manufactured dry penicillin sensitivity discs. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, pp. 898-905.
7. GREENBERG, L.; FITZPATRICK, K. M., AND BRANCH, A.: The status of the antibiotic disc in Canada, *Canad. M.A.J.* 76:194, 1957.
8. BRANCH, A.; STARKEY, D. H., AND POWER, E. E.: Control of antibiotic sensitivity discs. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, pp. 107-110.
9. KAYE, S.; IRMINGER, H. F., AND PHILIPS, C. R.: Ethylene oxide as a sterilizing agent for penicillin and streptomycin, *Bact. Proc.* 1951, p. 35.
10. KIRBY, W. M. M.; YOSHIHARA, G. M.; SUNSTED, K. S., AND WARREN, J. H.: Clinical usefulness of a single disc method for antibiotic sensitivity testing. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, pp. 892-897.
11. ENGLE, F. B., AND BASS, J. A.: The comparative antibiotic resistance of airborne microorganisms isolated from hospital areas. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, pp. 634-639.
12. VACCARO, H.; PAREDES, L., AND VALENZUELA, E.: In vitro sensitivity of 300 strains of *Micrococcus pyogenes* isolated from carriers to seven antibiotics. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, pp. 623-633.

# The Performance under Standardized Conditions of Antibiotic Sensitivity Tests Using Discs That Meet Official Canadian Standards

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In many papers by our group and other workers in the antibiotic field, it has been shown that there is widespread use of dry antibiotic sensitivity discs in the United States and Canada,<sup>1-4</sup> and that a substantial variation in the results of such tests may occur in using discs of different United States manufacturers and also different batches of the same manufacturer. Governmental controls have been introduced in Canada requiring that all discs be suitably labeled with a control number for each batch and that the assayed potency of the disc fall within 40 per cent plus or minus the labeled potency.<sup>5</sup> We have urged also that official requirements should be extended to define adequate concentrations for each type of disc so that performance tests would also meet standard requirements for outlining various degrees of sensitivity and resistance.<sup>6</sup> We have been informed in a personal communication that controls will shortly be introduced in the United States covering both assayed potency and performance of discs. Details of the Food and Drug Administration's proposed regulations have just come to hand and will be referred to in the discussion.<sup>7</sup> The reasons for advocating stricter controls will be brought out in the present work where the tests were performed with the majority of discs meeting present requirements of the Canadian Food and Drug Act.

A wide variety of discs were obtained in order to cover all possible variables, such as type of matrix (paper and tablet), concentrations of antibiotics, method of interpretation of results, and employment of one or more discs. These discs consisted of (1) American discs, the majority of which complied with the requirements of the Canadian Food and Drug Act, (2) Canadian discs, noncommercial and prepared as standard, and (3) European discs, which had been assayed as to antibiotic content but had not necessarily passed the Canadian requirements. Three countries were represented, France, Sweden, and Denmark.

## METHODS

The methods previously described have been followed.<sup>8-10</sup> Tryptose agar with thiamine (Difco) was the medium employed; the thickness of the agar in the plates was kept constant, a standard inoculum was used, and the plate was spread with a bent platinum wire in a manner to ensure uniform growth.

## INTERPRETATION OF RESULTS

The manner of recording results by the disc test is to outline three categories, i.e., resistant, moderately resistant, and sensitive. All American discs and the Canadian are manufactured and distributed in two or three discs of low and high potencies. The standardized methods used by us are interpreted as in table I.

The categories are defined as related to therapy; that is, infections due to sensitive organisms should respond to the usual dosages of the antibiotics; moderately resistant, to large doses; and resistant, show no response.<sup>11</sup>

TABLE I  
*Methods of Interpretation of Results*

Antibiotic	Tube dilution			Agar well		
	Sensitive	Moderately resistant	Resistant	Sensitive	Moderately resistant	Resistant
Penicillin	0.25, 1	2.5, 5, 10	>10	0.25, 1	5, 10, 25	>25
Chloramphenicol	5, 10	25, 50, 100	>100	10, 50	75, 100, 500	>500
Tetracycline	0.25, 1	5, 10, 25	>25	1, 5	10, 25, 50	>50

#### THE AMERICAN DISCS

The results are interpreted according to the instructions of the manufacturer.

*Penicillin.* Manufacturer 3: heavy inoculum. Sensitive means a marked zone of inhibition around high or low discs; moderately sensitive, minimal, if any, detectable zone around low and distinct zone around high; resistant, absence of zone of inhibition around both.

Manufacturer 4: heavy inoculum for staphylococci, three discs of varying concentration. Very sensitive means a zone of inhibition around all discs; sensitive, absence of zone around low disc; slightly sensitive, zone around high; resistant, no zones present. With light inoculum, discount zones of 12 mm. or repeat with heavy inoculum.

Manufacturer 5: Presence of a zone of inhibition around the disc indicates susceptibility and absence indicates resistance. The low concentration disc is to be tested first.

Manufacturer 6: Distinct zone of inhibition indicates susceptibility. The width is of no significance. They recommend using the low disc first.

Manufacturer 7: Zone of inhibition 8 to 12 mm. in diameter by 1 unit tablet indicates sensitivity; less than 8, the organism is relatively resistant; a zone of more than 12 mm., very sensitive; resistant, 7 to 9 zone around 10 unit tablet.

It will be seen from this that the method of interpretation advised by each manufacturer varies. We have attempted to follow these instructions, although in every case the instructions are not very specific. Also, in case our inoculum may not be as heavy as specified by manufacturers 3 and 4, we discount zones of 12 mm. or less around the discs.

*Chloramphenicol.* For manufacturers 3, 4, 5, and 6, interpretations of results for this antibiotic are similar to those for penicillin.

TABLE II  
*Summary of Interpretations*

		Penicillin	Chloramphenicol	Tetracycline
Manufacturer 9, disc size 6 mm.	Resistant	< 11 mm.	< 10 mm.	< 8 mm.
	Slightly sensitive	12-20 mm.	11-15 mm.	9-16 mm.
	Moderately sensitive	21-30 mm.	16-21 mm.	17-20 mm.
	Very sensitive	> 31 mm.	> 22 mm.	> 21 mm.
Manufacturer 8, disc size 11 mm. (one set of interpretations for all antibiotics)		Resistant	No zone	
		Moderately resistant		
		Sensitive		
Manufacturer 10, tablets, size 10 mm. (one set of interpretations for all antibiotics)			< 20 mm.	
			> 20 mm.	
		Resistant	< 15 mm.	
		Relatively resistant	16-22 mm.	
		Moderately sensitive	23-24 mm.	
		Sensitive	> 25 mm.	

TABLE III  
*Assay of Discs for Antibiotic Content*

Manufacturer	No. of discs		Potency in units		Control number
			Labeled	Assayed	
<i>Penicillin</i>					
American	3	2	2, 10	1.5, 9.9	Marked
	4	3	2,* 5,* 10	0.26, 2.3, 6.2	Marked
	5	2	1.5,* 5	3.4, 4.1	Marked
	6	2	1.5, 10	1.2, 11.8	Unmarked
	7	2	1, 10	1.0, 9.9	Marked
European	8	1		1.1	Marked
	9	1		11.2	Unmarked
	10	1	25	18.0	Unmarked
Canadian	11	3	2, 5, 10	2.5, 5.8, 10.5	
<i>Chloramphenicol</i>					
American	3	2	5, 30	4.6, 38.3	Marked
	4	3	5,* 10, 30	7.9, 10.8, 29.7	Marked
	5	2	10, 50	14.7, 34.1	Marked
	6	2	10, 50	11.6, 30.2	Unmarked
	7	2	2, 20	2.1, 16.0	Marked
European	8	1		48.3	Marked
	9	1		29.7	Unmarked
	10	1	1000	904	Unmarked
<i>Tetracycline</i>					
American	3	2	5, 30	4.8, 24.8	Marked
	4	3	5, 10, 30*	5.4, 10.5, 17.5	Marked
	5	2	10, 50	11.8, 59.9	Marked
	6	2	10, 50	8.4, 37.0	Unmarked
	7	2	10, 100	11.6, 123	Marked
European	8	1		2.9	Marked
	9	1		17.1	Unmarked
	10	1	1000	908	Unmarked

\* Did not fulfill Canadian requirements.

Manufacturer 7: Zone of inhibition 8 to 12 mm. in diameter by 2  $\mu$ g. tablet indicates sensitivity; less than 8, the organism is relatively resistant; a zone of more than 12 mm., very sensitive; resistant, 7 to 10 mm. zone around 20  $\mu$ g. tablet.

*Tetracycline.* For manufacturers 3, 4, 5, and 6, interpretations of results for this antibiotic are similar to those for penicillin.

Manufacturer 7: Zone of inhibition 8 to 12 mm. in diameter by 2  $\mu$ g. tablet indicates sensitivity; less than 8, the organism is relatively resistant; a zone of more than 12 mm., very sensitive; resistant, 7 to 10 mm. zone around 100  $\mu$ g. tablet.

In the case of European discs, a single disc, or tablet, is used and the sensitivity is gauged by the diameter of the zone of inhibition.<sup>12,13</sup> These interpretations are summarized in table II.

For purposes of uniformity, in other tables, for manufacturers using four categories of reporting, the moderately sensitive and very sensitive classes have been combined as sensitive, and the slightly sensitive is placed in our category of moderately resistant.

#### EXPERIMENTAL

All discs were assayed for antibiotic content by the Laboratory of Hygiene and the results are shown in table III. Unless marked by asterisk, they fulfill the Canadian requirements.

TABLE IV

*Comparison of Results of Dry Penicillin Discs—Canadian, American, and European  
—with Standard Methods*

Staphy- lococci	Source of disc										
	Standard*		American					European			Canadian
	1	2	3	4	5	6	7	8	9	10	11
P7	S	S	S 28 34	S 28 31 34	S 30 32	S 27 35	S 26 32	S	S	S	S
P9	S	S	S 28 35	S 28 31 35	S 21 26	S 32 33	S 27 35	S	S	S	S
P10	S	S	S 28 32	S large	S large	S 28 32		S	S	S	S
P19	S	S	S 13 38	S 28 34 36	S 32 35	S 31 40	S 26 33	S	S	S	S
P20	S	S	S 27 35	S 28 32 35	S large	S 28 34	S 28 35	S	S	S	S
P22	S	S	S 28 36	S 27 32 33	S large	S 27 35		S	S	S	S
P23	S	S	S 30 35	S 27 32 35	S large	S 27 35	S 23 30	S	S	S	S
P2	MR	MR	R — 10	R — 10 11	MR — 12	S 11 12	MR — 10	R	R	R	R
P3	MR	S	S 14 18	MR 13 15 18	S 15 15	S 14 16		MR	MR	MR	S
P4	MR	MR	MR 12 15	MR 12 14 16	S 12 15	S 14 15	S 10 14	MR	MR	MR	MR
P8	MR	MR	MR 11 15	MR 11 14 15	S large	S 14 15	S 11 13	MR	MR	MR	MR
P14	MR	S	MR 10 14	MR 11 13 15	S large	S 14 17		MR	MR	MR	MR
P16	MR	MR	MR 12	R — 11	R	S 10 11	R	R	R	R	R
P17	MR	MR	MR 12 16	MR — 11 13	S 15 16	S 13 11	MR — 11	MR	MR	MR	MR
P24	MR	MR	R — 10	R — 11	R	S 11 11	R — 9	R	R	MR	R
P25	MR	MR	R — 10	R — 12	R	S 10 —	R — 9	R	R	R	R
P1	R	MR	R — 11	R — 12	R	S 12 12	MR — 11	R	R	MR	R
P5	R	MR	MR — 13	R	R	MR — 11	R	R	MR	R	R
P6	R	MR	R — 11	R — 10	R	S 12 10		R	R	MR	R
P11	R	MR	R — 12	R — 11	R	MR — 11		R	MR	MR	R
P12	R	MR	R — 12	MR — 13	R	S 11 10	R	R	MR	MR	R
P13	R	MR	MR 9 14	R — 11	R	MR — 11	MR — 12	R	MR	R	MR
P15	R	MR	R — 11	R — 10 11	R	MR — 11	R	R	R	R	R
P18	R	R	R — 10	R — 12	R	MR — 11	R	R	R	MR	R
P21	R	MR	R — 10	R — 10	R	S 10 10	MR — 10	R	R	R	R

S = sensitive; MR = moderately resistant; R = resistant.

\* 1. Agar well. 2. Tube dilution.

TABLE V  
Summary of Table IV: Penicillin

Manufacturer	Interpretation		
	S	MR	R
1	7	9	9
2	9	15	1
3	8	7	10
4	7	6	12
5	12	1	12
6	20 (7)	5 (13)	0 (5)*
7	7	5	7 (19 strains)†
8	7	5	13
9	7	9	9
10	7	11	7
11	8	5	12

\* Figures in parentheses are our interpretations by zone size and not following the manufacturer's instructions.

† Supply of discs became depleted.

*Penicillin.* None of these penicillin discs gave zones of inhibition when tested against a strain of *Escherichia coli*.

The test organisms were 25 strains of coagulase-positive staphylococci, which, by the agar well method, showed seven sensitive, nine moderately resistant, and nine resistant. All but four organisms (no. 3, 6, 11, 22) were tested for penicillinase production. These all produced penicillinase with the exception of 17 and 19, which failed to produce any.

The interpretations of the results of tests using the various discs are listed in table IV and a summary of the numbers in each category in table V.

In the sensitive category, as defined by standardized methods, all the discs indicated sensitivity also. In the other two categories, there was disagreement, the results ranging from sensitive to resistant. A possible explanation of some of these discrepancies will be attempted by analyzing the results of the discs of particular manufacturers (see table V).

In one case, no. 9, there was complete numerical agreement. This was accomplished by a single disc containing 11.2 units by assay, interpreting the sensitivity judged from the size of the zone of inhibition. This had been determined by preliminary work with one grade paper and one concentration of antibiotic.

In contrast, although the disc of manufacturer 6 met the Canadian requirements for labeled potency, no correlation was achieved. This clearly indicates that, unless there are impurities present, a paper with too great releasing properties is being employed for the concentration used. The alternative would be to use smaller amounts of antibiotic or to change the recommended method of interpretation by using zone sizes to indicate categories of resistance and sensitivity. If this were done on the same basis as that of manufacturer 9, the results would be more comparable to the standardized method. (See table V, parentheses for change in interpretation.)

*Chloramphenicol.* The test organisms were staphylococci, which by both standard methods showed 11 sensitive, two resistant, and seven moderately resistant strains.

The interpretations of the results of the tests are listed in table VI and the numbers in each category in table VII.

The sensitive organisms showed up as such with all the discs except no. 7,

TABLE VI  
Comparison of Results of Dry Chloramphenicol Discs—Canadian, American, and European—with Standard Methods

Organism	Source of disc									
	Standard		American					European		
	1	2	3	4	5	6	7	8	9	10
C2	S	S	S	S	S	S	MR	S	S	S
C19	S	S	S	S	S	S	MR	S	S	S
C20	S	S	S	S	S	S	MR	S	S	S
C1	S	S	S	S	S	S	MR	S	S	S
C26	S	S	S	S	S	S	MR	S	S	S
C7	S	S	S	S	S	S	MR	S	S	S
C4	S	S	S	S	S	S	MR	S	S	S
C15	S	S	S	S	S	S	MR	S	S	S
C23	S	S	S	S	S	S	MR	S	S	S
C34	S	S	S	S	S	S	MR	S	S	S
C33	S	S	S	S	S	S	S	S	S	S
C28	MR	MR	MR	R	R	MR	R	R	MR	MR
C29	MR	MR	MR	MR	MR	MR	MR	MR	S	S
C21	MR	MR	MR	MR		S	R	R	R	MR
C5	MR	MR	R	MR		S	R	R	R	MR
C30	MR	MR	MR	MR		MR	R	R	R	S
C12	MR	MR	R	MR		MR	R	R	R	R
C13	MR	MR	R	R		R	R	R	R	MR
C27	R	R	R	R	R	R	R	R	R	R
C32	R	R	MR	MR	R	MR	R	R	R	MR

where obviously this tablet contained too little antibiotic for the matrix chosen. However, it met the Canadian requirements for labeled potency. In the resistant category, some organisms were reported moderately resistant, while in the moderately resistant category, there were some sensitive and some resistant. In two of the European discs (8 and 9) the intermediate zone is not well outlined. Tablet 10, although containing 904  $\mu\text{g.}$ , determines the three zones.

*Tetracycline.* In this batch of test strains there were 24 organisms: two gram-negative bacilli and 22 staphylococci. These included nine sensitive, 13 resistant, and two moderately resistant strains by the agar well method. The tube dilution method showed up more in the moderately resistant than resistant category as several organisms were sensitive to the highest concentration, i.e., 25  $\mu\text{g./ml.}$ , and were thus borderline strains.

The results of the tests are shown in table VIII and the summary in table IX.

It will be noted that there is good agreement in the disc methods except in the

TABLE VII  
Summary of Table VI: Chloramphenicol

Manufacturer	Interpretation		
	S	MR	R
1	11	7	2
2	11	7	2
3	11	5	4
4	11	6	3
5	11	1	3 (15 strains)*
6	13	5	2
7	1	11	8
8	11	1	8
9	12	1	7
10	13	5	2

\* Supply of discs became depleted.

TABLE VIII

*Comparison of Results of Dry Tetracycline Discs—Canadian, American, and European—with Standard Methods*

Organism	Source of disc									
	Standard		American					European		
	1	2	3	4	5	6	7	8	9	10
TT8	S	S	S	S		S	S	S	S	S
TT9	S	S	S	S		S	S	S	S	S
TT2	S	S	S	S		S	S	MR	MR	S
TT3	S	S	S	S		S	S	S	S	S
TT10	S	S	S	S		S	S	S	S	S
TT17	S	S	S	S		S	S	MR	S	S
TT4	S	S	S	S	S	S	S	S	S	S
TT23	S	S	S	S			S	MR	S	S
TT32	S	S	S	S	S		S	MR	S	S
TT18	MR	MR	MR	R			R	R	MR	MR
TT24	MR	MR	R	R		R	R	R	MR	MR
TT34	R	MR	R	R			R	R	MR	MR
TT15	R	R	R	R			R	R	MR	MR
TT7	R	R	R	R			R	R	MR	MR
TT12	R	MR	R	MR			R	R	MR	MR
TT13	R	R	R	R			R	R	MR	R
TT19	R	R	R	R			R	R	MR	R
TT1	R	R	R	R			R	R	MR	MR
TT16	R	R	R	R			R	R	MR	R
TT21	R	R	R	R	MR	R	R	R	MR	MR
TT5	R	R	R	R		R	R	R	MR	MR
TT30	R	MR	R	R			R	R	R	MR
TT25	R	MR	MR	R	R		R	R	MR	MR
TT35	R	MR	R	R	MR		R	R	MR	MR

case of manufacturer 8. The possible reason for this is that this disc contained only 2.9  $\mu\text{g}$ . The discs of manufacturers 9 and 10 both failed to show as many resistant strains as the others in spite of the fact that 9 contained 17.1  $\mu\text{g}$ . and 10 (a tablet), 908  $\mu\text{g}$ .

## DISCUSSION

These experiments, illustrated by the three examples below, show that the control of labeled potency alone, as practiced in Canada, is not sufficient to ensure a

TABLE IX

*Summary of Table VIII: Tetracycline*

Manufacturer	Interpretation		
	S	MR	R
1	9	2	13
2	9	7	8
3	9	2	13
4	9	1	14
5	2	2	1 (5 strains)*
6	7	—	3 (10 strains)*
7	9	—	15
8	5	4	15
9	8	15	1
10	9	12	3

\* Supply of discs became depleted.

particular disc giving the required accurate result in in vitro sensitivity tests. (1) The penicillin disc of manufacturer 6: The majority of organisms tested by this disc were sensitive instead of moderately resistant or resistant, in spite of the fact that labeled potency met the required official standard. Discs of this manufacturer are not marketed in Canada since no control numbers are marked on the label. (2) The chloramphenicol disc of manufacturer 7: The assayed potency of the low disc was the same as the labeled, but failed to distinguish any sensitive organisms. The concentration in this tablet was too low to give the required result. (3) The tetracycline disc of manufacturer 8: This contained 2.9  $\mu\text{g.}$  by assay and gave too few sensitive results. This disc was not labeled as to potency by the manufacturer.

For more complete control, some form of performance test is required since the concentration in a disc may vary greatly and still satisfactorily outline categories of sensitivity, provided the right matrix and the correct method of interpretation are used. This is illustrated in the case of chloramphenicol discs with assayed potencies varying from 4.6 to 904  $\mu\text{g.}$  In interpreting the tests with these two discs, the size of the zone of inhibition is not considered with the 4.6  $\mu\text{g.}$  disc, while with the 904  $\mu\text{g.}$  disc, the size of the zone of inhibition is measured and taken into account in assessing the degree of sensitivity.

Where zone size is used for interpretation, the manufacturers adopt different means, i.e., no. 10 retains the same zone size for resistance for all antibiotics by varying the concentration in the disc to fulfill this requirement; no. 9 varies the size of zone for each antibiotic. A very ingenious method of Hoette and Struyk,<sup>14</sup> of Holland, is to regulate the antibiotic content in each individual disc so that a zone of inhibition of 10 mm. corresponds to the usual blood levels obtained by ordinary dosage. In this way, they hope to attain clinical correlation with in vitro reporting. As far as we can ascertain, clinical correlation with presently reported in vitro tests still offers a challenge to demonstrate a close relationship to response of therapy. The three remaining problems are to define (1) the maximum variation between labeled potency and assayed potency for each antibiotic that can be allowed so as to guarantee a satisfactory performance test, (2) the type of performance test, and (3) clinical correlation.

*Limits of Potency.* The present Canadian requirements are plus or minus 40 per cent for all discs and have not been especially defined for each antibiotic and each type of matrix. Certain observations can be made from our tests on the performance of those discs that did not meet these requirements, without drawing any conclusions. (1) Penicillin, manufacturer 5, low disc: The assayed potency was 3.4 units instead of the labeled 1.5 (maximum allowed 2.1). The performance test resulted in 12 organisms being reported sensitive instead of 9. Manufacturer 4, low and medium discs: The low, labeled 2 units, assayed 0.26. This satisfactorily outlined the sensitive organisms. The medium disc, labeled 5 units, assayed 2.3 and gave no conclusive evidence of performance as the high disc was up to potency. (2) Tetracycline, manufacturer 4: The high disc, labeled 30, assayed 17.5  $\mu\text{g.}$  (18 allowed). The results of performance tests were good. It seems to us that this problem of outlining limits of potency will depend on the type of matrix used. If a single matrix were employed by all manufacturers, the limits of potency would readily be definable. In the present state, where heterogeneous matrices are used, the limits may vary for each manufacturer and possibly each antibiotic. However, the Canadian requirements have, for the most part, improved the quality of the discs.

*Type of Performance Test.* The test used here differs from that outlined by the Food and Drug Administration's committee recommendations in that the size of the zone of inhibition is employed as test of potency, while we have tested 25 strains of organisms of known degrees of sensitivity.

*Clinical Correlation.* It has been the aim of our clinical laboratories to make the term "resistant" synonymous with lack of response to therapy and "sensitive" with response to therapy. Such a correlation can never be attained until the whole system of in vitro sensitivity testing is standardized. The present control of discs is a definite advance in achieving this goal. The actual amounts of the antibiotics in the discs may require changing, in order to achieve standard results with all manufacturers' discs, unless they all use the same matrix.

The recommendations of the Food and Drug Administration to the manufacturers of discs include the following points: Use paper of comparable grade, size, and shape; antibiotics used should be free of potentiating substances; there should be uniformity of impregnation of disc; assay should average 67 to 150 per cent labeled potency and be controlled by a performance test based on zone size; in the uniformity tests, discs of a certain batch should show no larger than 2.5 mm. variation in zone size from the controls; the higher potency disc must be no less than 2.5 times the lower; expiration dates and storage conditions must be marked on the package of discs.

It remains to be seen whether the controls for marketing antibiotic sensitivity discs as recommended by the Food and Drug Administration will adequately outline the three categories of sensitivity and resistance. Also, it is hoped that the paper chosen for the discs will fail to show the small zones of inhibition that sometimes occur with penicillin and are said to be of no significance. This appears to be possible since such zones are not seen with discs from at least one manufacturer. If clinical correlation, using the interpretation of tests with the controlled discs, is not satisfactory, it will be necessary to change the method of interpretation.

#### SUMMARY

Discs from various American and European sources were tested against strains of bacteria whose antibiotic sensitivities had previously been determined by two standard methods that have given reproducible results in 10 Veterans Hospitals in Canada for some years.

All batches of discs used were assayed for potency by the official control laboratory (Biologics Control Laboratory, Food and Drug Division, Department of National Health and Welfare, Ottawa).

Evidence is presented indicating that control of potency alone is not sufficient to guarantee uniformity of reporting in terms of "sensitive," "moderately resistant," and "resistant."

Reasons are given for advocating additional efforts toward standardization, including the standardization of techniques and of matrix for discs, plus performance testing and clinical correlations.

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## BIBLIOGRAPHY

1. BRANCH, A.; STARKEY, D. H., AND POWER, E. E.: A correlation of various antibiotic in vitro sensitivity tests. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, p. 407.
2. BRANCH, A.; STARKEY, D. H.; POWER, E. E., AND GREENBERG, L.: Problems of standardization of manufactured dry penicillin sensitivity discs. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 898.
3. HOFFMAN, R. V.; JACKSON, G. G., AND TURNER, M. S.: Reliability of antibiotic sensitivity tests as determined by a survey study, *J. Lab. & Clin. Med.* 51:873, 1958.
4. KIRBY, W. M. M.; YOSHIHARA, G. M.; SUNDSTED, K. S., AND WARREN, J. H.: Clinical usefulness of a single disc method for antibiotic sensitivity testing. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc. 1957, p. 892.
5. GREENBERG, L.; FITZPATRICK, K. M., AND BRANCH, A.: The status of the antibiotic disc in Canada, *Canad. M.A.J.* 76:194, 1957.
6. BRANCH, A.; STARKEY, D. H., AND POWER, E. E.: Control of antibiotic sensitivity discs. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 107.
7. WELCH ET AL: The Results of a Survey Made by the Food and Drug Administration with Commercial Batches of Antibiotic Diagnostic Discs and Tablets. The Assay and Control of Antibiotic Sensitivity Discs, U.S.A. Food and Drug Administration, 1958. Personal communication.
8. BRANCH, A., AND POWER, E. E.: Tube dilution method for bacterial sensitivities to antibiotics as standardized for D.V.A. hospital laboratories, *Canad. D.V.A. Treat. Serv. Bull.* 8:378, 1953.
9. STARKEY, D. H., AND GREGORY, E.: Agar well method for bacterial sensitivities to antibiotics as standardized for D.V.A. hospital laboratories, *Canad. D.V.A. Treat. Serv. Bull.* 8:381, 1953.
10. POWER, E. E.: Use and abuse of antibiotic sensitivity tests, *Canad. J.M. Tech.* 17:2, 1955.
11. RODGER, K. C.; BRANCH, A.; POWER, E. E.; STARKEY, D. H.; GREGORY, E.; MURRAY, R. D., AND HARROP, J.: Antibiotic therapy: correlation of clinical results with laboratory sensitivity tests, *Canad. M.A.J.* 74:605, 1956.
12. LUND, E.: Resistance determinations with tablets containing various amounts of active substances, *Acta path. et microbiol. Scandinav.* 33:278, 1953.
13. ERICSSON, H.; HÖGMAN, C., AND WICKMAN, K.: A paper disk method for determination of bacterial sensitivity to chemotherapeutic and antibiotic agents, *Scandinav. J. Clin. & Lab. Investigation* 6:23, suppl. II, 1955.
14. HOETTE, I., AND STRUYK, A.: A modified method for evaluation of clinical usefulness of antibiotics, *J. Lab. & Clin. Med.* 51:638, 1958.

# The International Situation with Regard to the Use of Discs for Antibiotic Sensitivity Tests

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Since its inception, the Antibiotic Control Laboratory of the Canadian Department of Veterans Affairs Hospitals has been attempting to maintain uniformity in the performance of sensitivity tests, as carried out in the 10 departmental hospital laboratories across Canada. Maintenance of uniformity through standard testing techniques has been very satisfactory for the past three years, but serious difficulties have arisen through the increasing use of discs of commercial manufacture.

Since controls over the labeled potency of antibiotic sensitivity discs were introduced in Canada more than a year ago, there has been some restoration of confidence among laboratory workers and some growth of appreciation of the necessity for standardizing techniques. If it can be firmly established that nearly all clinical laboratories are using commercial discs, control and standardization of such discs would seem to be the first step on which we should concentrate. Concomitantly, we should continue to stress the need for standardizing techniques. It may be hoped that international acceptance of standardization will follow.

In the present paper, the discussion will be limited to the knowledge we have been able to acquire by means of a questionnaire on the subject of antibiotic sensitivity tests. For Canada, there has been full coverage, while some replies have been received from representative bacteriologists in Europe and other parts of the world.

The questionnaire was sent to more than 150 clinical laboratories of all sizes across Canada, and 70 replies, representing all provinces, have been received, to date. Analysis of the returns shows that only two laboratories are not using discs but depend on the tube dilution method. The method of choice in one other hospital was the agar well, but this was supplemented by discs. All the other hospitals report the use of discs of varying potencies as routine. These are obtained from three American manufacturers, with only one laboratory using European tablets. However, there are individual variations in their usage, as follows: (1) the products of one manufacturer were employed in 39 hospitals, while those of two or three different manufacturers in the others; (2) in six instances, homemade discs (mostly penicillin) supplemented the commercial; (3) a single disc was used (in some cases it was the high and in others the low potency disc); (4) discs of one manufacturer were consistently employed for some antibiotics and those of another for others; and (5) the use of the tube dilution method to supplement the disc results was reported from nine hospitals.

Another fact brought out from the survey was that a multiple type disc is more popular than individual discs in Canada. This preponderance is accounted for by its more extensive use in the western provinces.

In answer to the specific question as to whether the interpretations of results were based on the size of the zone of inhibition, 47 of 70 replied in the negative. The question regarding the necessity of in vitro sensitivity tests being standardized resulted in 90 per cent agreement that such is desirable.

Many general comments were volunteered. For example, erroneous results were held to be due to lack of standardization of methods as well as to discs, and,

TABLE I  
*Antibiotic Sensitivity Tests Used in Various Countries*

	Discs or tablets					
	Commercial		Home-made	Tube dilution	Agar well	Miscellaneous
	Indigenous manufacture	Foreign				
Canada		American, Danish	x	x	x	
Czechoslovakia	x	(?)	x			Cylinder
Denmark	x					
England	x	American	x	x	x	Ditch, cylinder
France	x		x	x	x	
Germany	x	American	x	x	x	
Holland		Danish	x	x		Agar dilution
Iran		American, French				
Ireland		English, American				
Norway		Swedish				
Scotland			x			
South Africa		American	x			
Spain		American, French	x			
Sweden	x					
Switzerland	x		x			
Turkey		American	x	x	x	Fish beads
United States	x	(?)	x	x	x	Gradient, cylinder
Yugoslavia		Danish	x		x	

specifically, the size of the inoculum was mentioned. Lack of faith in the discs, as presently manufactured, was expressed. The method was used as a rough guide, since it is easy to perform and numerous tests can be done in a short time. Other opinions expressed were that a single disc was inadequate and that a device in the shape of a cogwheel or ring, containing a number of antibiotics impregnated at various points, was undesirable. One individual suggested it was not worth wasting too much time on standardizing discs which, at best, give only an approximation of degrees of sensitivity. Another opposing point of view was that, for accuracy, a standard disc should be available from a governmental laboratory as a standard control. It was also suggested that standard organisms be made available for circulation.

In summary, from the replies to the Canadian questionnaire, it is obvious that different types of commercial discs are extensively used for antibiotic sensitivity tests and that there is a majority opinion that standardization of methods would improve the results of the laboratory tests.

In the United States the questionnaire has not been circulated, but, from publications and papers read at the Annual Antibiotics symposia, one can also conclude that discs are in wide use there.

Regarding the situation in overseas countries, it is indicated from a few replies to the questionnaire and from correspondence with workers in the antibiotic field that, besides homemade discs, there are several countries where the commercial disc method is chiefly in use. In Europe, these are procurable from commercial firms or well-known national institutes for research in the country concerned. In France, for example, the Pasteur Institute is almost the sole supplier of discs, and some of these Pasteur discs are imported into Spain and Iran. In Denmark, the Roskilde Medical Company sells tablets, and these are used chiefly in that country but also elsewhere, e.g., Holland, Canada, and Yugoslavia. Swedish discs of the Karolinska Sjukhuset Company are distributed extensively in that country and Norway (table I).

Table I also shows that other methods, such as the ditch, cylinder, agar well, and

tube dilution, are used in England. In the Edinburgh area of Scotland as well as in South Africa, homemade discs are the choice. The replies to the questionnaire from foreign countries, as those from Canada, agreed that international standardization of discs and the method of performing sensitivity tests is highly desirable.

A list of the names of manufacturers of antibiotic sensitivity discs, known to us, is appended for reference.

#### SUMMARY AND CONCLUSIONS

From the results of answers to a questionnaire distributed to all laboratories in Canada, from papers published in the United States, and from correspondence with antibiotic centers in Europe and other parts of the world, it can be stated that the disc method of performing antibiotic sensitivity tests is the one most generally employed. For this reason, we feel that the key to the problem of gaining international standardization of antibiotic sensitivity tests lies in acceptance of standards for the manufacturing of discs and also for the whole method in detail. A start was made on the governmental control of discs in Canada over a year ago and is being initiated in the United States. It remains to be seen whether these controls can be proved adequate and can be enforced. If so, international acceptance should follow. Such a step, leading to more uniform reporting of the results of antibiotic sensitivity tests, would place the clinician in a more confident position for choosing the right antibiotic in treating his patient and for comparing results of therapy in different centers.

#### MANUFACTURERS OF ANTIBIOTIC SENSITIVITY DISCS

Roskilde Medical Co., Ltd., Roskilde, Denmark.

Evans Medical Supplies Ltd., Liverpool-London, England.

Pasteur Institute, 28 Rue Du Dr. Roux, Paris 15e, France.

Fa. Mack, Illertissen Bayern, Bavaria, Germany.

Karolinska Sjukhuset, Stockholm 60, Sweden.

Bürgerspital, Basel, Switzerland.

Baltimore Biological Laboratory Inc., 1640 Gorsuch Ave., Baltimore 18, Maryland, U.S.A.

Consolidated Laboratories, Inc., Box 234, Chicago Heights, Illinois, U.S.A.

Difco Laboratories, 920 Henry Street, Detroit 1, Michigan, U.S.A.

National Bio Test Corporation, Box 1713, Benson Station, Omaha, Nebraska, U.S.A.

Reed and Carnrick, Pharmaceuticals, 155 VanWagenen Ave., Jersey City 5, New Jersey, U.S.A.

# Antibiotic Sensitivity Using Pretreated Plates

## II. A Demonstration of Inhibitory Activity with a Low Level Combination of a Sulfonamide and Polymyxin B against *Proteus* Species

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The use of an effective combination of antibiotic and chemotherapeutic substances in vivo is often overlooked or limited by the lack of an appropriate visual demonstration of in vitro activity. The use of broth in tubes is usually unsatisfactory and the rapid growth of many species of gram-negative bacteria on the surface of agar plates prevents visible interactions between slowly diffusing substances when applied as impregnated discs after inoculation. A previous report<sup>4</sup> outlined a method whereby antibiotic or chemotherapeutic sensitivity test discs, when allowed to diffuse through agar in plates before the test culture was added, showed increases of zone inhibition diameters of 25 to 125 per cent on sensitive organisms over those tested directly. In the past three years some 4000 clinical specimens were tested for antibiotic sensitivity patterns by the dipped polymyxin B cogwheel method,<sup>3</sup> and of these approximately 5 per cent showed the presence of *Proteus* species with the resultant overgrowth of the entire plate. However it was noted that, when any sulfonamide disc was included in the test pattern around the polymyxin B cogwheel, most strains of *Proteus* showed a visible response in the area circumscribed by the intimate diffusion of these two substances. This report presents experimental evidence of the in vitro inhibitory activity of various levels and combinations of sulfonamides and polymyxin B against swarming strains of the *Proteus* species.

### MATERIALS AND METHODS

*Media.* Trypticase soy agar and broth (BBL) was used throughout the experiment.

*Inoculum.* A strain of *Proteus vulgaris* isolated from a chronic kidney infection was kept on Trypticase soy agar slants, transferred to broth and incubated 3 to 4 hours and added to cooled agar, 1 ml./100 ml. bottle, just before pouring seed layers.

*Test Materials.* Polymyxin B stock solution .2 per cent or 20,000 units/ml. was made by adding 10 ml. sterile distilled water to 20 mg. sterile polymyxin B powder in 20 ml. vials, stored in the refrigerator and diluted as required. Sulfathiazole and sulfamethoxypyridazine stock solution were made up with the sodium salt as a 2 per cent solution with distilled water, sterilized by autoclaving and diluted as required. Impregnated discs were used as commercially supplied in 1 and .25 mg./disc of the sulfonamides and 50 and 300 units of polymyxin B, respectively. Lower levels were made by dipping sterile blank discs of the same size and composition into appropriate dilutions of sterile solutions with an average uptake of 0.05 ml./disc.

### METHODS

Trypticase soy agar dispensed in 100 ml. amounts in 4 oz. screw cap bottles with polymyxin B and sulfonamide added as needed (tables I to IV) was enough to pour five Petri plates with 16 ml. base layer and 4 ml. seed layer. Since many plates showed zones of inhibition in excess of 60 mm. only two discs were used per plate.

TABLE I

*Polymyxin B Disc 50 Units on Trypticase Soy Agar with Added Sulfamethoxypyridazine*

Sulfonamide in base, $\gamma$ /ml.	Direct incubation	24 hour diffusion, mm.	48 hour diffusion, mm.
80	12	22	35
40	12	18	22
20	12	17	30
8	12	16	24
4	12	14	15
2	Trace	10	12
Control	Negative	Negative	Negative

Plates not directly incubated were held in the refrigerator for varying periods of time to permit maximum diffusion from the discs into surrounding area of the treated agar. All plates were incubated at 37 C. for 24 hours and zone diameters measured in mm.

## RESULTS

Polymyxin B and the two sulfonamide compounds tested in this experiment continued to diffuse through nutrient agar from a wet or dry impregnated disc for at least 72 hours (tables I to IV).

Since neither substance alone appears to be active at high levels, the direct effect of very low levels, as noted from the zone sizes, would indicate an extremely toxic reaction through their combined activity against viable *Proteus* cells. Although the discs made up with sulfonamide drugs carry 30 to 50 times the amount of active material (250 and 1000 gamma/disc) as does the polymyxin B disc (5 to 30 gamma/disc) yet the activity against *Proteus* in the agar of each is visible when activated by the other member of the team at the 1 and 2 gamma/ml. levels.

When the plates are held for a few days at room temperature following a diffusion and incubation period, growth toward the disc is often visible only on the surface from the edges of the zone area. Continued diffusion and absorption by growing cells may so lower the amount of drugs present that normal growth is again possible. The evolution of resistant variants must not be overlooked; however, to date, no naturally resistant mutant has been isolated.

Sulfonamide compounds, while not interfering with growth except at high concentrations, do appear to reduce the motility not only of *Proteus* species but also many other gram-negative organisms with active flagella.

TABLE II

*Sulfamethoxypyridazine Discs 250  $\gamma$  on Trypticase Soy Agar with Added Polymyxin B*

Polymyxin B in base, $\gamma$ /ml.	Direct incubation, mm.	24 hour diffusion, mm.	48 hour diffusion, mm.
40	28	53	66
20	28	53	64
10	26	52	64
4	24	49	64
2	Trace	40	58
1	Trace	20	25
Control	Negative	Negative	Negative

TABLE III  
*Polymyxin B Discs on Trypticase Soy Agar Sulfathiazole in Base*

Sulfonamide in base, γ/ml.	Polymyxin B discs, units	Direct incubation, mm.	4 hour diffusion, mm.	24 hour diffusion, mm.	48 hour diffusion, mm.	72 hour diffusion, mm.
2	50	8	10	13	10	10
	300	15	15	20	24	28
8	50	11	12	15	18	18
	300	17	20	26	28	32
Control	50	0	0	0	0	0
	300	0	0	0	0	0

#### DISCUSSION

The growth habits of *Proteus* species under extremely varied conditions has provided it with remarkable powers of adaptation and also endowed it with a high level of resistance to most antibiotic substances. However, the addition of two dissimilar substances at one time may involve multiple mechanisms. The presence of polymyxin B may increase the permeability of the cells and so render the mucopolysaccharide substrate more readily accessible to the action of an enzyme (or a drug, such as sulfathiazole).<sup>5,6</sup>

The excellent control of motility and growth of highly active *Proteus* strains in vitro by very small levels of polymyxin B and sulfonamides should warrant a carefully controlled in vivo study. Swedish workers<sup>1</sup> have shown that *Proteus* infections are spread from patient to patient by rectal thermometers, bedding, and once established in the intestine they tend to remain there and eventually set up infections in the genitourinary tract.

While sulfonamides have been used extensively in chronic urinary tract infections it is desirable to have a sulfonamide compound that is not easily acetylated in the liver nor readily precipitated in the highly acid conditions in the kidney. The addition of small doses of polymyxin B by intramuscular injection at regular intervals should provide as active an anti-*Proteus* combination in vivo as it does in vitro, since levels of sulfonamide in the urine often exceed 1000 gamma/ml.<sup>2</sup> while polymyxin B may reach 50 to 80 gamma/ml. during treatment.

Nevertheless, since urinary tract infections with *Proteus* species are difficult to eradicate, a complete in vitro study is always indicated to determine the most active combination of a sulfonamide or other compound and polymyxin B that would be capable of controlling the infection.

TABLE IV  
*Sulfathiazole Discs on Trypticase Soy Agar Polymyxin B in Base*

Polymyxin B in base, γ/ml.	Sulfonamide /disc, γ	Direct incubation, mm.	4 hour diffusion, mm.	24 hour diffusion, mm.	48 hour diffusion, mm.	72 hour diffusion, mm.
2	250	20	30	40	55	70
	1000	20	31	50	65	80
8	250	32	37	40	65	70
	1000	35	40	50	75	80
Control	250	0	0	0	0	0
	1000	0	0	0	0	0

A low level of a polymyxin B-sulfonamide combination has been shown to be highly specific for the in vitro inhibition of *Proteus* species.

The visible interaction between antibiotics and chemotherapeutic compounds in agar plates, layer seeded with test organisms, may be greatly enhanced by a 24 to 72 hour diffusion period in the refrigerator prior to incubation.

## BIBLIOGRAPHY

1. EDEBO, L., AND LAURELL, G.: Hospital infections of urinary tract with *Proteus*, Acta path. et microbiol. Scandinav. 43:93-106, 1958.
2. GOODMAN, L. S., AND GILMAN, A.: The pharmacological basis of therapeutics, Chapter 56, The Macmillan Company, New York, 2nd Edition, 1956.
3. HERMAN, L. G.: Polymyxin B: Further studies on its potential in the clinical laboratory. In: Antibiotics Annual 1956-1957. New York, Medical Encyclopedia, Inc., 1957; pp. 1026-1030.
4. HERMAN, L. G.: Antibiotic sensitivity using pretreated plates. Preliminary report. In: Antibiotics Annual 1957-1958. New York, Medical Encyclopedia, Inc., 1958; pp. 111-113.
5. NEWTON, B. A.: The properties and mode of action of the polymyxins, Bact. Rev. 20:10-27, 1956.
6. WARREN, G. H.; GRAY, J., AND YURCHENKO, J. A.: Effect of polymyxin on the lysis of *Neisseria catarrhalis* by lysozyme, J. Bact. 74:788-793, 1957.

# Slide Microculture in Bacteriological Diagnosis and Antibiotic Screening

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The purpose of this report is to describe a method for the rapid growth and identification of bacteria and the application of this technique for rapid testing of antibiotic sensitivity.

The increasing prevalence of serious clinical infections owing to antibiotic-resistant bacteria emphasizes the need for rapid bacteriological diagnosis. The speedy selection of appropriate therapeutic agents is of primary importance in the control of such infections. The frequent complication of changing microbial flora and antibiotic sensitivity during therapy requires repeated culturing and sensitivity determinations for precise and successful management.

Methods currently available for cultural diagnosis and sensitivity testing are limited by the 12 to 18 hours necessary for macroscopic growth to appear on solid media. Antibiotic sensitivity testing in liquid media is more rapid but requires pure cultures; mixed cultures obtained directly from infectious material cannot be routinely tested in this fashion.

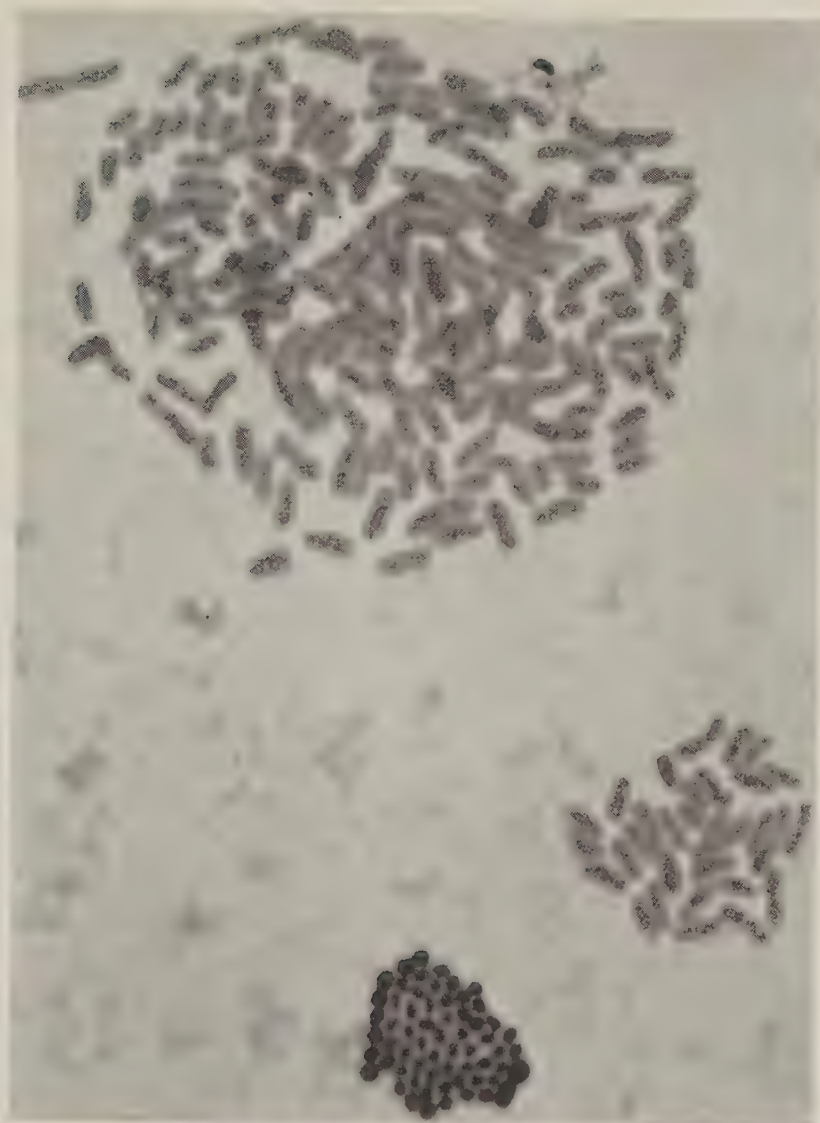
A microculture technique for the growth of *Mycobacterium tuberculosis* was first described by Koch<sup>1</sup> in 1882. In 1941, Pryce<sup>2</sup> described a practical modification of this slide culture technique for *Myco. tuberculosis*. In this method, the specimen is smeared on a slide, which is then placed in a test tube of nutrient liquid medium and the whole incubated. Since then, numerous investigators have demonstrated that this is a useful and rapid method for the cultivation and antibiotic sensitivity testing of the tubercle bacillus. However, a search of the literature reveals only one attempt to utilize the method of Pryce for the cultivation of bacteria other than *Myco. tuberculosis*. In 1951, Chen and Chang<sup>3</sup> described their unsuccessful attempts to modify Pryce's technique for routine bacteriological purposes.

A different technique was developed independently by Frost<sup>4,5</sup> and described in 1916 and 1921. Since these original reports, no further applications of this technique have been discovered in a review of the literature. The methods detailed in this paper are modifications of Frost's original technique.

In principle, the method allows the cultivation of bacteria in thin films of agar on glass microscope slides. Preliminary microscopic identification of mixed cultures, by means of Gram stain and microcolony characteristics, may be accomplished after four hours of incubation. By incorporating antibiotic agents into the agar medium, antibiotic sensitivity testing may be carried out simultaneously, in duplicate microcultures. Inhibition of growth or actual bacteriolysis is determined by comparison with the antibiotic-free control microculture.

This technique is simple and reproducible. It is carried out as follows. Infectious material, either obtained directly from the patient or from pure cultures, is taken up on a broth-moistened sterile swab and smeared on the surface of a warm, sterile, glass microscope slide. At one end of this slide is placed a small (0.03 ml.) drop each of melted tryptic digest agar at 43 C. and Tryptose phosphate broth. Using a second sterile glass slide, these two drops are mixed and then drawn as a film over the surface of the first slide, upon which the specimen has been smeared. For anti-

FIG. 1. Photomicrograph of slide microculture showing colonies of *Proteus vulgaris* and *Micrococcus pyogenes* var. *aureus*. Gram stain. (X 900)



biotic sensitivity testing the agent(s) to be tested are incorporated, in appropriate concentration, into the Tryptose phosphate broth, and duplicate slide microcultures are set up as described. The slide cultures are then incubated in a humidified atmosphere at 37 C. for four hours. Then they are air dried at room temperature and stained with Gram stain in the usual fashion. Further fixation is not necessary. Direct microscopic examination, using "high dry" or oil magnification, shows characteristically stained bacterial cells in microcolonies (fig. 1). The effect of incorporated antibiotics is easily ascertained by comparisons of colony size and cellular morphology between the antibiotic-containing cultures and the antibiotic-free cultures.

Studies in this laboratory have shown that a number of bacteria of clinical importance can be cultivated by this method. These include streptococci, micrococci, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Aerobacter aerogenes*, *Diplococcus pneumoniae*, *Escherichia coli*, *Salmonella*, and *Proteus* species. Characteristic cellular and colonial morphology of these organisms allows for preliminary microscopic diagnosis, which can be confirmed later by the usual techniques. Studies of

TABLE I  
*Comparison of 218 Antibiotic Sensitivity Tests, Slide/Tube*

Organism (no. of strains)	Antibiotic, $\mu\text{g.}/\text{ml.}^a$							
	Strepto- mycin (8)	Chloram- phenicol (8)	Tetra- cycline (8)	Erythro- mycin (8)	Baci- tracin (8)	Novo- biocin (8)	Penicil- lin (2.5)	Poly- myxin (8)
<i>E. coli</i> (10)	9/9	9/9	9/10			1/1	1/1	
<i>Proteus</i> (10)	8/9	8/9	10/10	1/1	1/1	8/9	2/2	
<i>A. aerogenes</i> (9)	8/8	7/8	8/9			2/2	1/1	
<i>Pseudomonas</i> (4)	4/4	4/4	4/4			3/3		3/3
<i>K. pneumoniae</i> (4)	4/4	4/4	4/4			1/1		
<i>Enterococcus</i> (3)	1/1	1/1	2/3	1/1	1/1	1/1	3/3	
<i>Staphylococcus</i> (17)	5/6	10/11	7/8	12/14	13/13	2/2	15/16	
<i>Streptococcus</i> (1)	1/1	1/1	1/1	1/1	1/1	1/1	1/1	

<sup>a</sup> Discrepancies were noted in 13 tests (6 per cent).

the sensitivity of the technique indicate that recognizable growth can be obtained with inocula containing 5000 organisms/cu. ml. and good growth with 25,000/cu. ml. Antibiotic sensitivity tests have been carried out against these organisms with all of the clinically used antibiotics. The results with this method correlate closely with simultaneous tube dilution sensitivity tests. Of 218 parallel tests, there were only 13 (6 per cent) discrepancies (table I). In all of these, the microculture method demonstrated inhibition of growth, whereas no inhibition was seen in the tube dilution method. This rapid microculture technique has proved useful in a number of clinical situations in which urgent diagnosis and selection of therapy were necessary. Thus, it has been used to clinical advantage in cases of staphylococcal pneumonia and pseudomembranous enterocolitis as well as in the complicated antibiotic therapy of burn wound sepsis.

#### SUMMARY

This paper reports the development of a technique for the rapid growth, identification, and antibiotic sensitivity testing of clinically important bacteria. Clinically infectious material or pure cultures of organisms are cultivated in thin films of agar on glass slides. After four hours of incubation, these microcultures are stained directly with Gram stain and observed microscopically. Characteristic colonial morphology, cellular pattern, and tinctorial characteristics are easily observed and allow for preliminary identification. Incorporation of antibiotics into the medium permits sensitivity testing, the results of which correlate closely with tube dilution sensitivity tests. Observations of the effect on bacterial colonial morphology and cellular structure of various antibiotics can be carried out. Investigations have indicated that this is a simple, reproducible technique, adaptable for routine clinical use in those situations in which speed in diagnosis and selection of therapy are necessary.

#### BIBLIOGRAPHY

1. KOCH, R.: Über die Aetiologie der Tuberkulose, *Klin. Wchnschr.* 19:221-230, 1882.
2. PRYCE, D. M.: Sputum film cultures of tubercle bacilli: A method for the early observation of growth, *J. Path. & Bact.* 53:327-334, 1941.
3. CHEN, H., AND CHANG, N.: Studies on the slide culture for common bacteria, *Chinese M. J.* 69:221-225, 1951.
4. FROST, W. D.: A rapid method of counting living bacteria in milk and other richly seeded materials, *J.A.M.A.* 66:889-890, March, 1916.
5. FROST, W. D.: A rapid cultural method of diagnosing diphtheria, *J.A.M.A.* 76:130, Jan., 1921.

# Qualitative and Quantitative Bacteriological Data: A Reliable Guide to the Selection of Candidates for, and Response to, Antibiotic Therapy in Urinary Tract Infections

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The clinician must ask himself: Is this patient a good candidate for antibiotic therapy? Is he responding satisfactorily to the regimen of antibiotic therapy instituted? His answers are partially guided by available bacteriological data. To what extent are bacteriological results a guide or even an aid to the clinician in the treatment of urinary tract infections? Frequently the isolation of organisms from catheterized or cleanly voided specimens presents a problem of interpretation not only to the bacteriologist but also to the clinician when the report is received. Many laboratories ignore the quantitative aspects in these reports, which can result in a dilemma for the physician who has to decide upon a course of antibiotic therapy. Thus, certain laboratories will tend to ignore a relatively few organisms initially isolated and render a specific interpretation for the physician, such as "no growth" or "contamination." In addition, many laboratories feel that routine quantitative isolations are too expensive and time consuming.

An attempt is made here to throw some light upon the matter from a practical viewpoint. Basically, one assumption is made with regard to values here reported, namely: the fact that all patients from whom urine specimens were submitted for bacteriological examination had a potential clinical disease entity, such as pyelonephritis, urethritis, cystitis, prostatitis, or combination of genitourinary pathology. The specimens submitted were taken before, during, and following antibiotic therapy, although not necessarily all three or more from the same patient. This paper is not an evaluation of the efficacy of a specific antibiotic or combinations thereof, but primarily an attempt to establish some relationship, if any, between the degree of pyuria present in an urine, the type and number of bacteria isolated, and the clinical disease entity. The degree of pyuria present was computed on the basis of the number of white blood cells per low-power field found in a catheterized or cleanly voided specimen. The organisms concerned in this study included *Staphylococcus aureus*, enterococci, *Escherichia coli*, *Aerobacter aerogenes*, *Pseudomonas aeruginosa*, and *Proteus* sp. The antibiotics concerned included penicillin, erythromycin, chlortetracycline, oxytetracycline and tetracycline, chloramphenicol, novobiocin, and oleandomycin, together with the chemotherapeutic agents nitrofurantoin and certain sulfonamides.

The physicians were furnished with sterile 8 dram screw cap vials for collection of urine specimens for culture, prior to which microscopic examinations for white blood cells were made. Specimens not processed within one hour were refrigerated until cultured. At no time were these specimens refrigerated for more than 20 hours (overnight). There was no deviation from the normal routine of initial inoculation: a 4 mm. loopful streaked onto brain-heart infusion blood agar and eosin methylene blue agar plates, and transfer of 0.5 to 1.0 ml. of urine to brain-heart infusion broth tube. In addition, plate counts were made at  $10^1$ ,  $10^3$ , and  $10^5$  dilutions on brain-heart infusion agar plates without blood. All media were incubated at 35 C. for 18 to 24 hours. Smears were made from all broth tubes to detect presence of viable organisms, particularly mixed flora, and also from plates when indicated, for purity. Typical colonies (1 to 3) were transferred to a small volume of

brain-heart infusion broth and incubated for two hours or until there was evidence of gross turbidity. These tubes were then used as the source of swab streaking to brain-heart infusion blood agar plates for antibiotic susceptibility testing by the agar diffusion technique. The plates inoculated for counting were read for actual number of viable organisms after 24 hours incubation. Blood plates and E.M.B. plates showing no growth after 24 hours were reinoculated from broth tube if there was growth here (verified by Gram stain examination). All broth tubes showing no growth after 24 hours were reincubated for an additional 24 hours before reporting that there was no growth.

No bacterial growth occurring in the liquid media after 24 hours incubation invariably resulted in no growth on plate media and also resulted in a bacterial count of 0 or less than 10 per ml. (no growth on any count plate). Associated with this was the absence of pyuria or at most a low-grade pyuria (less than 5 white blood cells per low-power field). A patient demonstrating such findings was considered not a likely candidate for antibiotic therapy. Such patients usually responded to symptomatic treatment on sulfonamide therapy. If patients were already under therapy, these findings might be interpreted as a favorable response to the regimen of antibiotic therapy instituted.

Growth occurring in liquid media (broth) but demonstrating no growth on solid media from the initial inoculation usually resulted in a bacterial count of less than 100/ml. The majority of these were less than 10/ml., many showing no growth whatever on the count plates. These bacterial findings correlated well with a low grade of pyuria (5 or fewer white blood cells). Organisms isolated in this category were usually of the gram-positive group (staphylococci and enterococci). These patients appeared to require a regimen of chemotherapy. Although they proved to be good candidates for antibiotic therapy and usually responded favorably, it was noted that, unless the specific antibiotic was reinforced with a broad-spectrum antibiotic, either concurrently or following apparent cure of the gram-positive infection, a fair number of patients demonstrated emergence of gram-negative organisms not originally detected in the laboratory. It was rare to encounter a gram-negative organism showing a low bacterial count. Counts with gram-negative organisms were usually greater than 1000/ml. and frequently in the millions per ml.

Table I presents a composite summary of laboratory findings as they occurred in the majority of the situations of disease entity. There appeared to be no quantitative relationship between bacteriological findings and the disease entity. This is more pointedly demonstrated in table II.

Bacteriological findings, especially quantitatively, do aid the doctor in confirming his diagnosis as well as in evaluating the efficacy of a specific regimen antibiotic therapy. This is particularly true with regard to the presence of gram-posi-

TABLE I  
*Summary of Bacteriological Data from Urinary Infections*

Liquid media	Solid media	Total count per ml.	Microscopic white blood cells per low-power field	Organism
0*	0	0	0 to 5	0
+†	0	0 to 10	<5	Gram-positive
+	+	<1,000	5-10	Gram-positive
+	+	>10,000	Variable	Gram-negative

\* 0 — no growth.  
† + — growth.

TABLE II  
Bacteriological Data from Patients with Urinary Infections

Lab. no.	Bacterial growth		Microscopic		Organism	Diagnosis
	Liquid media	Solid media	Count	White blood cells per low-power field		
1951	0	0	0	3-4	—	Papillary urethritis
1989	0	0	0	"rare"	—	Pyelonephritis
1991	0	0	0	1-2	—	Papillary urethritis
1996	0	0	0	"occasional"	—	Postoperative prostatectomy pyuria
2005	0	0	0	8-10	—	Tuberculous cystitis
1909	0	0	0	0	—	Urethrocystitis
1910	0	0	0	0	—	Hemorrhagic cystitis
1917	0	0	0	6-8	—	Subacute prostatitis
1956	+	0	<10	2-3	Enterococcus	Urethritis
1929	+	0	<10	"a few"	Enterococcus and <i>Gaffkeya tetragena</i>	Urethrocystitis
1935	+	0	<10	2-3	Enterococcus and occasional <i>Micrococcus</i>	Chronic prostatitis with prostatic calculi
1944	+	0	<10	2-4	A few yeasts only	Pseudomembranous trigonitis and chronic urethritis
1941	+	0	<10	"clear"	<i>Proteus mirabilis</i>	Chronic cystitis
1949	+	0	<10	2-4	Enterococcus	Pyelonephritis, left
1964	+	0	<10	2-3	Enterococcus	Urethral stricture & urethrocystitis
1999	+	0	<10	"pyuria persists"	Diphtheroids only	Urethral stricture & urethrocystitis
2013	+	0	<10	1-2	<i>Proteus mirabilis</i>	Urethral stricture & urethrocystitis
1967	+	0	<10	3-4	<i>Streptococcus zymogenes</i>	Chronic urethrocystitis
1973	+	0	<10	1-2	<i>Escherichia coli</i>	Hemorrhagic cystitis
1980	+	0	<10	1-2	<i>Streptococcus zymogenes</i>	Prostatitis & seminal vesiculitis
1984	+	0	<10	8-10	Occasional <i>Micrococcus</i> and diphtheroids	Chronic cystitis
1987	+	0	<10	"clear"	<i>Staphylococcus aureus</i>	Postoperative transurethral resection pyuria
1988	+	0	<10	not stated	<i>Staphylococcus aureus</i>	Hydronephrosis, postoperative pyeloplasty
1990	+	0	<10	"occasional"	Diphtheroids only	Chronic urethroprostatitis
1992	+	0	<10	"occasional"	<i>Proteus morgani</i>	Cystitis cystica
1997	+	0	<10	4-5	Enterococcus	Chronic urethrocystitis, pyelonephritis
2008	+	0	<10	1-2	<i>Staphylococcus aureus</i>	Essential hematuria
2012	+	0	<10	4-5	<i>Proteus mirabilis</i>	Chronic pyelonephritis with urethral stricture
2017	+	0	<10	2-4	<i>Streptococcus zymogenes</i>	Urethrocystitis, atrophic vaginitis
2018	+	0	<10	2-3	Occasional <i>Micrococcus</i>	Right renal calculus
2020	+	0	<10	4-5	A few enterococci	Chronic urethritis
2024	+	+	<10	"clear"	<i>Staphylococcus aureus</i>	Urethritis
1906	+	0	<10	"loaded"	<i>Gaffkeya tetragena</i>	Urethrocystitis
1932	+	0	<10	12-20	<i>Gaffkeya tetragena</i>	Cystitis
1969	+	0	20	2-3	<i>Aerobacter aerogenes</i>	Pyelonephritis, left
1979	+	+	50	"negative"	<i>Streptococcus zymogenes</i> , <i>Pseudomonas aeruginosa</i>	Subacute cystitis with bladder diverticulum
2016	+	0	50	3-5	<i>Staphylococcus aureus</i>	Chronic interstitial cystitis
2021	+	0	50	3-4	<i>Staphylococcus albus</i>	Chronic urethritis
2022	+	0	70	4-5	Enterococcus	Right urethral stricture and pyelonephritis
1959	+	+	90	"a few"	<i>Proteus morgani</i>	Urethrocystitis
1938	+	+	420	3-4	<i>Proteus mirabilis</i>	Chronic urethritis
1968	+	+	700	5-6	<i>Aerobacter aerogenes</i> & <i>Streptococcus zymogenes</i>	Urethral stricture and urethrocystitis
1955	+	0	750	2-3	<i>Neisseria sicca</i>	Chronic prostatitis with prostatic calculi
1939	+	+	1,900	not stated	<i>Staphylococcus albus</i> , 25% diphtheroids	Phimosis with low-grade urethrocystitis
2019	+	+	2,430	10-20	<i>Escherichia coli</i> and <i>Aerobacter aerogenes</i>	Postoperative prostatectomy pyuria
1976	+	+	5,220	2-3	<i>Escherichia intermedium</i>	Postoperative transurethral resection pyuria
2029	+	+	7,920	1	<i>Aerobacter aerogenes</i>	Urethrocystitis, postoperative cystitis
1961	+	+	14,000	20	<i>Aerobacter aerogenes</i>	Urethrocystitis
1994	+	+	19,000	4-5	Enterococcus	Chronic cystitis with cystocele
2004	+	+	21,000	5-6	<i>Aerobacter aerogenes</i>	Carcinoma of prostate with cystitis
1963	+	+	27,000	5-10	<i>Aerobacter aerogenes</i>	Postoperative transurethral resection pyuria
1978	+	+	30,000	2-4	<i>Neisseria sicca</i> (plus 80 enterococci)	Chronic prostatitis with prostatic calculi
1983	+	+	37,000	8-10	<i>Aerobacter aerogenes</i> & <i>Proteus mirabilis</i>	Postoperative prostatectomy pyuria
1998	+	+	43,000	2-3	<i>Neisseria sicca</i> + 2000 <i>Staphylococcus aureus</i>	Chronic prostatitis with prostatic calculi
1952	+	+	44,000	4-5	<i>Proteus mirabilis</i>	Chronic urethritis
1965	+	+	50,000	5-6	<i>Pseudomonas aeruginosa</i>	Chronic cystitis with bladder diverticulum
1930	+	+	65,000	"loaded"	<i>Aerobacter aerogenes</i>	Urethrocystitis
2031	+	+	75,500	20	<i>Alkaligenes faecalis</i>	Chronic recurrent lower urinary tract infection

Table II Continued on Page 846

TABLE II (Continued)  
Bacteriological Data from Patients with Urinary Infections (continued)

Lab. no.	Bacterial growth		Microscopic		Organism	Diagnosis
	Liquid media	Solid media	Count	White blood cells per low-power field		
1977	+	+	89,000	10-12	<i>Escherichia coli</i>	Papillary carcinoma of bladder with chronic cystitis
2002	+	+	114,000	8-10	<i>Aerobacter aerogenes</i>	Chronic cystitis
1970	+	+	122,000	10-20	<i>Aerobacter aerogenes</i>	Urethrocystitis, postoperative cystitis
1911	+	+	168,000	4-8	<i>Proteus mirabilis</i>	Chronic cystitis
1953	+	+	265,000	"occasional"	<i>Escherichia coli</i>	Phimosis with low-grade urethroprostatitis
2000	+	+	289,000	0	<i>Proteus morganii</i>	Cystitis, recurrent
2001	+	+	300,000	10-20	<i>Aerobacter aerogenes</i>	Postoperative transurethral resection pyuria
2007	+	+	376,000	8-10	<i>Escherichia coli</i>	Ureteral stricture, left
2010	+	+	409,000	<5	<i>Aerobacter aerogenes</i>	Urethrocystitis, postoperative cystitis
1907	+	+	560,000	"loaded"	<i>Escherichia coli</i>	Urethrocystitis
1995	+	+	585,000	"loaded"	<i>Escherichia coli</i>	Urethritis
1993	+	+	705,000	"many"	<i>Proteus rettgeri</i>	Benign prostatic hypertrophy with prostatic calculi & prostatitis
1931	+	+	760,000	4-9	<i>Escherichia coli</i>	Chronic cystitis with obstructing prostate
1958	+	+	780,000	not stated	A few gram-positive cocci & <i>Aerobacter aerogenes</i>	Urethral stricture & urethrocystitis
2009	+	+	850,000	5-6	<i>Pseudomonas aeruginosa</i>	Subacute prostatitis
1966	+	+	1,200,000	"loaded"	<i>Aerobacter aerogenes</i>	Chronic cystitis with benign prostatic hypertrophy
1947	+	+	5,700,000	not stated	<i>Pseudomonas</i> sp.	Chronic cystitis with bladder diverticulum
2003	+	+	7,800,000	1-2	<i>Aerobacter aerogenes</i>	Urethrocystitis
2023	+	+	10,200,000	"many"	<i>Aerobacter aerogenes</i>	Chronic cystitis
1945	+	+	11,600,000	"loaded"	<i>Proteus mirabilis</i>	Chronic pyelonephritis
1981	+	+	13,700,000	20-30	<i>Klebsiella pneumoniae</i>	Bladder diverticula, renal calculus
1942	+	+	14,200,000	"loaded"	A few gram-positive cocci, <i>Aerobacter aerogenes</i> , <i>Escherichia</i> & <i>Pseudomonas</i> sp.	Urethrocystitis, postoperative cystitis
1908	+	+	25,800,000	"loaded"	<i>Aerobacter aerogenes</i>	Urethrocystitis
1962	+	+	32,000,000	10-12	<i>Escherichia coli</i>	Chronic pyelonephritis
1960	+	+	78,500,000	20	<i>Escherichia intermedium</i> & 1/3 <i>Streptococcus zymogenes</i>	Chronic recurrent lower urinary tract infection
2011	+	+	113,000,000	"loaded"	<i>Aerobacter aerogenes</i>	Chronic recurrent lower urinary tract infection

tive organisms, usually few numerically, in contrast to the incidence of a high number of gram-negative organisms. Each group has potentially a similar clinical significance. The majority of organisms isolated in the urinary tract infections are aerobic and failure to grow on solid media within 24 hours usually exclude gram-negative organisms. *Proteus rettgeri* and *Proteus morganii*, however, at times require a longer period of incubation. In mixed infections such findings usually exclude gram-negative organisms as the primary organism. This does not preclude the potential significance of a few gram-positive organisms.

The incidence of a high bacteriological count of gram-positive organisms is rare. In this situation growth in liquid but not on solid media coincides well with a low degree of pyuria and confirms well the clinical impression or diagnosis by the physician, whether it is before, during, or following therapy. Prolonged refrigerated storage of collected urine specimens prior to processing (longer than 24 hours) apparently has little significant bearing on the quantitative recovery of gram-negative organisms. However, prolonged storage of urine specimens containing a low count of gram-positive organisms can significantly affect their recovery, which may necessitate additional specimens.

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## I. Effect of Methods on Apparent Bactericidal Concentrations

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There are a number of reports in the literature on the bactericidal properties of various antibiotics, and this property is often considered an important characteristic of the antibiotic. The basic principles involved in determining the disinfectant (bactericidal) action of antimicrobial substances are generally well known, in regard to substances other than antibiotics. These principles are extensively discussed by Wilson and Miles.<sup>1</sup> With respect to the determination of the bactericidal action of antibiotics, however, a wide variety of procedures have been employed,<sup>2-14</sup> some of which do not adequately consider the basic principles pertinent to this type of study. The methods employed by various workers with antibiotics have varied from the viable cell count procedure of Jawetz et al<sup>7</sup> to the retransfer sterility determination on a broth dilution bacteriostatic test series of tubes, described by Waisbren.<sup>5</sup> Many modifications derived from these two procedures have been used and the data thus obtained reported in the literature. It would appear that no uniform method of approach to the problem of determining the bactericidal action of antibiotics is generally employed.

In the course of extensive studies in our laboratories on the bactericidal properties of ristocetin, we observed that different procedures and even slight modifications of a procedure gave different apparent bactericidal concentrations with a single bacterial strain. This observation led us to a critical evaluation of bactericidal test procedures. The preliminary results of this study indicate that the variation in apparent bactericidal concentrations of an antibiotic, due to different methods, is of such significance that the results of bactericidal determinations are meaningful only if the details of the procedure employed are defined. We felt that a report of these preliminary results would be of general interest to those engaged in studies of this type; we also felt that it would be worthwhile to draw the attention of investigators in the field to the importance of the methodology employed in bactericidal determinations with antibiotics.

### METHODS AND MATERIALS

*Procedures for the Bactericidal Tests.* Our studies on methods were concerned chiefly with variations based on bacteriostatic tube dilution procedures. Surviving cells were determined by plating in agar media. We were interested in developing a method that gives both the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) with a reasonable degree of reproducibility and accuracy.

Method 1 was a modified retransfer technique, in which the surviving cells were estimated by plate counts. A series of duplicate tubes of 5 ml. of Tryptose phosphate broth, containing the antibiotic in twofold increments from subinhibitory levels to 200  $\mu\text{g.}/\text{ml.}$ , were employed. The tubes were inoculated with 0.1 ml. of a 1:100 dilution of an 18 hour Tryptose phosphate broth culture. Data accumulated

in our laboratory show that this gives 100,000 cells/ml. or greater in the inoculated tubes. The organisms were passed through two broth cultures from a refrigerated agar slant to prepare inoculum. This inoculum procedure was standard in all methods studied. Tubes in which no growth appeared after 48 hours' incubation at 37 C. were cultured for surviving cells. Two 0.1 ml. aliquots from each tube were plated in Petri dishes, using Tryptose phosphate agar. The plates were incubated 72 hours at 37 C. Longer incubation did not significantly alter the counts. The minimum bactericidal concentration was considered to be the antibiotic concentration at which 99.9 per cent of the cells were killed.

Method 1-A was identical, except that brain-heart infusion broth was employed for the tube dilution portion of the test.

Method 2 was the same as method 1, except for one added feature. The test was carried out in screw cap tubes, and the tubes were vigorously shaken after 2, 4, and 6 hours' incubation.

Method 2-A was identical to method 2, except that brain-heart infusion broth was used for the tube dilution part of the test.

of 50 ml. flasks was prepared, containing 20 ml. of brain-heart infusion broth carrying between 50,000 and 100,000 bacterial cells/ml. The desired number of cells was obtained by appropriate dilutions of an 18 hour broth culture in the same medium. Antibiotic was added, the flasks were incubated at 37 C., and, at prescribed intervals (usually after 3, 6, 24, and 48 hours), plate counts were made of all flasks. Sampling was limited to 0.5 ml. at each time interval. A tryptone, yeast extract, beef extract, and glucose medium was used for the plate counts, since it accentuated the orange pigment of *Staphylococcus aureus*. Plates were incubated for 72 hours at 37 C. The flasks were shaken vigorously before each plating. Sterility tests were carried out on the flasks showing no growth after 48 hours' incubation. One-half ml. quantities of the medium from each flask were subcultured in 15 ml. of fluid thioglycollate medium. The tubes were incubated for three or more days at 37 C. We did not consider sterility tests significant when they were made from flasks containing more than 50  $\mu$ g./ml. of ristocetin, since antibiotic carryover might be inhibitory to small inocula. A 99.9 per cent killing of the cells in 48 hours was considered a significant bactericidal action.

Method 4 employed continuous agitation of the tubes during incubation. Screw cap tubes, 20 by 150 mm., containing 10 ml. of brain-heart infusion broth, were incubated on a reciprocal shaker operating at 76 cycles/min., with a stroke of three inches. The tubes were placed in a horizontal position. The inocula and the various concentrations of antibiotic were added in the same manner as described with the previous methods. Plate counts were made after 3, 6, 24, and 48 hours' incubation at 37 C. Sample size was limited to 0.5 ml. for each time interval, in order to minimize the reduction of volume of the medium. Sterility tests were made after 48 hours in the manner described for method 3.

*Bacterial Strains.* Although bactericidal tests were carried out with numerous strains of staphylococci, data on only 9 strains are presented as follows: *Staphylococcus albus*, Romansky 3519, isolated from acute bacterial endocarditis, resistant to penicillin; *Staphylococcus aureus* 682, isolated from enterocolitis, resistant to penicillin, erythromycin, the tetracyclines, streptomycin, and chloramphenicol; *Staph. aureus* 698, isolated from a thigh infection, resistant to penicillin; *Staph. aureus*, Wise J-391,<sup>15</sup> resistant to penicillin; *Staph. aureus*, Wise J-306,<sup>15</sup> resistant to penicillin, the tetracyclines, streptomycin, and chloramphenicol; *Staph.*

*aureus*, Wise J-591,<sup>15</sup> resistant to the tetracyclines and streptomycin; and *Staph. aureus*, Romansky 892, 893, and 894,<sup>16</sup> resistant to penicillin, erythromycin, the tetracyclines, and streptomycin.

## RESULTS AND DISCUSSION

Early in our studies, we became convinced that the retransfer technique employing a sterility test<sup>5</sup> and the streak plate method<sup>6</sup> were not sufficiently quantitative for our needs. The sterility test gives no measure of numbers of surviving cells, and, furthermore, the size of the sample cultured and the care with which it is cultured can greatly affect the apparent bactericidal concentration. The streak method, while giving an estimate of numbers of surviving cells, is limited in accuracy, since a loopful of culture is not a constant volume and is too small a sample for the precision we desired.

The selection of 50,000 to 100,000 cells/ml. for our quantitative procedures was an arbitrary decision. We found that in the range of 25,000 to 200,000 cells, with strains of staphylococci and the antibiotic ristocetin, results were reasonably consistent. Also, we believe this is a reasonable approximation of the *in vivo* situation with a systemic infection. Larger numbers of cells have frequently been used.<sup>5,8</sup> The limited data we obtained in this regard indicated that larger numbers of cells tend to increase the apparent minimum bactericidal concentration.

Antibiotics usually show a weaker bactericidal activity against resting cells than against multiplying cells.<sup>17</sup> In this study, we found that resting cells were significantly less sensitive to ristocetin. Since we were dealing with an antibiotic with proved *in vivo* activity, the resting cell technique was not considered a desirable procedure. After studying several media, we decided on a rich medium, brain-heart infusion, in which nearly all staphylococci and many other bacteria grow profusely. This medium is widely used in medical bacteriology for the tube dilution test. The difference in the MBC with a strain of *Staph. albus* due to the medium is shown in table I. Brain-heart infusion (method 1-A) gave an MBC of 100  $\mu\text{g./ml.}$ , in contrast to an MBC of 200  $\mu\text{g./ml.}$  obtained with Tryptose phosphate broth (method 1).

Shaking of the system during the early part of the incubation period had a very marked effect on the bactericidal activity. The results in table I are typical of our data with the coagulase-negative staphylococci and illustrate this point. The apparent MBC with a stationary tube system was 100  $\mu\text{g./ml.}$  (method 1-A). Shaking the tubes after 2, 4, and 5 hours' incubation reduced the MBC to 12.5  $\mu\text{g./ml.}$  (method 2-A). The flask method (method 3) and the continuously shaken method (method 4), which are quantitative procedures, gave results similar to method 2-A. However, with method 4, a few more surviving cells were found at 12.5  $\mu\text{g./ml.}$  than were found with method 3. The cells can properly be considered "persisters,"<sup>18,19</sup> for we were unable to isolate colonies with any increased resistance. We feel, on the basis of the data presented in table I, that method 4 gave the most meaningful information on the bactericidal action of ristocetin against *Staph. albus* 3519.

Agitation of the culture tubes did not usually show so great an effect with the coagulase-positive strains of staphylococci. However, several strains were found that showed an effect similar to that shown in table I. In table II are shown data typical of strains of *Staph. aureus*. The problem of surviving cells is evident with methods 1 and 2. The rather indefinite MBC of methods 1 and 2 is contrasted with the sharp end points of methods 3 and 4. Tubes apparently sterile were seldom observed with

TABLE I  
*Bactericidal Action of Ristocetin Against Staphylococcus albus 3579*

Method	Time, hours	Antibiotic concentrations, $\mu\text{g./ml.}$										MIC, $\mu\text{g./ml.}$	MBC, $\mu\text{g./ml.}$
		0	3.1	6.2	12.5	25	50	100	200				
1	48	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	< 10			12.5	200
1-A	48	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	> 3,000	28	< 10			12.5	100
2-A	48	> 3,000	> 3,000	> 3,000	< 10	< 10	< 10	< 10	< 10			12.5	12.5
3	0	95,000	95,000	95,000	95,000	95,000	95,000	95,000					
	6	$4 \times 10^6$	206,000	23,300	4,150	4,150	2,950					6.25	
	24	$550 \times 10^6$	$350 \times 10^6$	122,500	< 100	< 100	< 100					12.5	
	48	$202 \times 10^6$	$150 \times 10^6$	$95.5 \times 10^6$	Approx. 50	Sterile	Sterile						12.5
4	0	156,000	156,000	156,000	156,000	156,000	156,000	156,000					
	6	$11.9 \times 10^6$	$1.34 \times 10^6$	124,000	3,000	2,800	3,450					12.5	
	24	$510 \times 10^6$	$620 \times 10^6$	$1.05 \times 10^9$	< 100	< 100	< 100					12.5	
	48	$163 \times 10^6$	$108 \times 10^6$	$5 \times 10^6$	170	Sterile	Sterile						25

TABLE II  
*Bactericidal Action of Ristocetin Against Staphylococcus aureus 682*

Method	Time, hours	Antibiotic concentrations, $\mu\text{g./ml.}$										MIC, $\mu\text{g./ml.}$	MBC, $\mu\text{g./ml.}$
		0	3.1	6.2	12.5	25	50	100	200				
1	48	> 3,000	> 3,000	> 3,000	> 3,000	90	65	25	20			12.5	25
2	48	> 3,000	> 3,000	> 3,000	> 3,000	85	130	60	40			12.5	25 or 100
3	0	54,000	54,000	54,000	54,000	54,000							
	6	$370 \times 10^6$	4,900	260	6,200	2,200						6.2	
	24	$545 \times 10^6$	$565 \times 10^6$	< 100	160	< 100						6.2	
	48	$1.38 \times 10^9$	$1.47 \times 10^9$	< 10	Sterile	Sterile						6.2	
4		Not sterile											
	0	77,000	77,000	77,000	77,000	77,000							
	6	$225 \times 10^6$	49,000	230	310	550						6.2	
	24	$455 \times 10^6$	$275 \times 10^6$	< 10	< 10	< 10						6.2	
	48	$225 \times 10^6$	$250 \times 10^6$	490	Sterile	Sterile							12.5

TABLE III  
*Bactericidal Action of Ristocetin Against Staphylococcus aureus 698*

Method	Time, hours	Antibiotic concentrations, $\mu\text{g./ml.}$										MIC, $\mu\text{g./ml.}$	MBC, $\mu\text{g./ml.}$
		0	3.1	6.2	12.5	25	50	100	200				
1	48	>3,000	>3,000	55	77	322	570	140	23			6.2	6.2 (zone)
2	48	>3,000	>3,000	65	58	197	302	600	85			6.2	6.2 (zone)
3	0	28,000	28,000	28,000	28,000	28,000	28,000	28,000	28,000				
	6	$12.4 \times 10^6$	8,500	8,250	10,000	16,500	17,500	19,000	19,000				
	24	$625 \times 10^6$	$1.3 \times 10^6$	Approx. 110	410	2,000	1,950	3,100	2,100			3.1	
	48	$880 \times 10^6$	$2 \times 10^9$	Approx. 40	< 10	< 10							
4	0	44,300	44,300		Not sterile	Not sterile	Approx. 40	< 100	< 100			6.2	12.5 (zone)
	6	$5.5 \times 10^6$	51,000	12,200	44,300	44,300	44,300	44,300	44,300				
	24	$195 \times 10^6$	$325 \times 10^6$	Approx. 70	12,200	23,000	28,500	38,000	38,000				
	48	$290 \times 10^6$	$390 \times 10^6$	< 10	Approx. 160	430	2,600	5,700	6,900			6.2	
				Not sterile	< 10	Approx. 30	< 10	Approx. 250	Approx. 350			6.2	6.2 (zone)
					Not sterile		Not sterile						

TABLE IV  
*Bactericidal Action of Ristocetin, Using Method 3*

Staphylococcus strain	Time, hours	Antibiotic concentrations, $\mu\text{g./ml.}$										MIC, $\mu\text{g./ml.}$	MBC, $\mu\text{g./ml.}$
		0	3.1	6.2	12.5	25							
Wise J391	0	71,800	71,800	71,800	71,800	71,800							
	6	$119 \times 10^6$	150,000	1,285	1,900	3,400							
	24	$650 \times 10^6$	$645 \times 10^6$	47,500	Approx. 10	Approx. 15						6.2	
	48	$1.48 \times 10^9$	$1.4 \times 10^9$	$600 \times 10^6$	Sterile	Sterile						12.5	12.5
Wise J306	0	36,300	36,300	36,300	36,300	36,300							
	6	$70 \times 10^6$	3,600	1,425	1,525	1,435							
	24	$740 \times 10^6$	$42.5 \times 10^6$	1,465	< 10	< 10						6.2	
	48	$460 \times 10^6$	$685 \times 10^6$	5,000	Sterile	Sterile						6.2	12.5
Wise J571	0	30,000	30,000	30,000	30,000	30,000							
	6	$48 \times 10^6$	17,550	Approx. 70	Approx. 195	Approx. 150						6.25	
	24	$365 \times 10^6$	$310 \times 10^6$	< 10	< 10	< 10						12.5	
	48	$645 \times 10^6$	$630 \times 10^6$	$310 \times 10^6$	Sterile	Sterile							12.5

TABLE V

*Bactericidal Action of Ristocetin, Using Method 4*

<i>Staphylococcus</i> strain	Time, hours	Antibiotic concentrations, $\mu\text{g./ml.}$					MIC, $\mu\text{g./ml.}$	MBC, $\mu\text{g./ml.}$
		0	3.1	6.2	12.5	25		
Romansky 892	0	40,200	40,200	40,200	40,200	40,200		
	6	$450 \times 10^6$	5,600	340	270	650		
	24	$1.2 \times 10^9$	$3.58 \times 10^9$	$< 10$	$< 10$	$< 10$	6.2	
	48	$290 \times 10^6$	$1.14 \times 10^9$	Sterile	Sterile	Sterile	6.2	6.2
Romansky 893	0	39,600	39,600	39,600	39,600	39,600		
	6	$410 \times 10^6$	11,800	655	700	1,200		
	24	$1.3 \times 10^9$	$2.6 \times 10^9$	$< 10$	$< 10$	$< 10$	6.2	
	48	$210 \times 10^6$	$225 \times 10^6$	Sterile	Sterile	Sterile	6.2	6.2
Romansky 894	0	53,100	53,100	53,100	53,100	53,100		
	6	$575 \times 10^6$	12,000	755	695	700		
	24	$895 \times 10^6$	$2.8 \times 10^9$	$< 10$	$< 10$	$< 10$	6.2	
	48	$225 \times 10^6$	$665 \times 10^6$	Sterile	Sterile	Sterile	6.2	6.2

methods 1 and 2, even at high concentrations of the antibiotic. In contrast, sterility was usually obtained with methods 3 and 4. Method 4 detected more persister cells than method 3, as was noted with *Staph. albus* 3519.

Among the various strains of staphylococci studied, we encountered one culture of *Staph. aureus* that consistently showed the zone phenomenon. The bacterial cells were killed more readily near the MIC than at higher concentrations of antibiotic. Since this culture was unusual, we studied it in some detail. The results are presented in table III. The zone phenomenon was evident with all methods, but method 4, after 24 and 48 hours' incubation, showed it most clearly. The results with method 3 were somewhat inconsistent at the 48 hour observation. It is apparent from results of this nature that sterility tests alone give little information as to bactericidal effect. The data from plate counts are of limited value also. However, the two tests combined give a reasonably clear picture of the presence and numbers of surviving cells. There is little doubt that these cells represent true persisters, since we were unable, in repeated experiments, to obtain cultures resistant to ristocetin.

We used methods 3 and 4 to study additional strains of staphylococci. The results with method 3 on three strains obtained from another laboratory are given in table IV. These strains were considered, on the basis of a stationary tube re-transfer technique,<sup>15</sup> to be resistant to the bactericidal action of ristocetin. All three strains were found, using method 3, to have an MBC identical with or close to their MIC.

Representative data obtained with method 4 are given in table V. These were also cultures that, by a different technique,<sup>16</sup> were not killed by ristocetin. The data in table V show a rapid bactericidal effect with sterility obtained in 48 hours or less. The numbers of cells were sharply reduced in six hours. In all cases, the MIC values at 24 and 48 hours' incubation were identical, which is not usually the case with stationary systems. A constantly agitated system (method 4) promotes rapid growth of the organisms, which permits the study of a greater portion of the growth cycle of a culture within a given time interval.

The marked variability in the results obtained with the different methods illustrate the effect of differences in technique and interpretation of data. This study points out six factors that are of prime importance in determining whether an antibiotic has bactericidal activity and at what concentration that activity is significant. These factors may be listed as follows: (1) dispersion of cells, allowing intimate contact of the antibiotic with susceptible cells; (2) the concentration of bacterial cells in the system; (3) the physiological state of the cells; (4) the method of determining the presence or estimating the number of surviving cells; (5) the time interval over which the observations are recorded; and (6) the criterion used to decide whether a bactericidal effect has been observed.

At the present state of our investigation, we believe that method 4 gives us the most meaningful information. Preliminary studies comparing the bactericidal activity of other antibiotics by method 4 are in progress. The results of these studies will be reported in subsequent publications.

#### SUMMARY AND CONCLUSIONS

The apparent minimum bactericidal concentration of the antibiotic ristocetin was demonstrated to be greatly influenced by the technique used. Studies were carried out on some of the variables, in order to develop a procedure that would

give reproducible and meaningful data. Strains of staphylococci were used for these studies.

Four methods of measuring bactericidal activity were compared. The preferred method is a continuously agitated system, with cells growing in a rich medium. The death rate of the cells was estimated by plate counts.

Data are presented on several strains of staphylococci to illustrate these methods.

#### BIBLIOGRAPHY

1. WILSON, G. S., AND MILES, A. A.: *In: Principles of Bacteriology and Immunity*, Baltimore, Williams and Wilkins Co., 1955.
2. ENGLISH, A. R.; FIELD, M. F.; SZENDY, S. R.; TAGLIANI, N. J., AND FITTS, R. A.: Magnamycin. I. In vitro studies, *Antib. & Chemo.* 2:678-688, 1952.
3. JAWETZ, E.; GUNNISON, J. B.; COLEMAN, V. R., AND KEMPE, H. C.: A laboratory test for bacterial sensitivity to combinations of antibiotics, *Am. J. Clin. Path.* 25:1016-1031, 1955.
4. HILSON, G. R., AND ELEK, S. P.: Routine testing of the bactericidal action of antibiotics for clinical purposes, *J. Lab. & Clin. Med.* 44:589-594, 1954.
5. WAISBREN, B. A.: In vitro activity against *Micrococcus pyogenes* of various combinations of antimicrobial agents, *J. Lab. & Clin. Med.* 46:583-591, 1955.
6. RANTZ, L. A.; RANDALL, E.; THUM, L., AND BARKER, L. F.: The effects of vancomycin, oleandomycin, and novobiocin on staphylococci in vitro, *Antib. & Chemo.* 7:399-409, 1957.
7. JAWETZ, E.; BERTIE, W., AND SONNE, M.: The participation of novobiocin and vancomycin in combined antibiotic action against staphylococci, *AM & CT* 4:40-44, 1957.
8. GUNNISON, J. B.; JAWETZ, E., AND COLEMAN, V. R.: The effects of combinations of antibiotics on enterococci in vitro, *J. Lab. & Clin. Med.* 36:900, 1950.
9. JACKSON, G. G., AND FINLAND, M.: Comparison of methods for determining sensitivity of bacteria to antibiotics in vitro, *A.M.A. Arch. Int. Med.* 88:446, 1951.
10. ZIEGLER, D. W.; WOLFE, R. N., AND MCGUIRE, J. M.: Vancomycin, a new antibiotic. II. In vitro antibacterial studies. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, p. 612.
11. GERACI, J. E.; HEILMAN, F. R.; NICHOLS, D. R.; WELLMAN, W. E., AND ROSS, G. T.: Some laboratory and clinical experiences with a new antibiotic, vancomycin. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 90.
12. STREITFELD, M. M.: The replica strip gradient plate technique for determination of synergism or antagonism of antibiotics paired in various concentration ratios: a bacteriostatic and bactericidal assay. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, p. 906.
13. HOBBY, G. L., AND LENERT, T. F.: Observations on the mode of action of oleandomycin, *Antib. & Chemo.* 8:219, 1958.
14. JENNINGS, J. C., AND OLSON, B. H.: The bactericidal action of synnematin B, *Antib. & Chemo.* 8:245, 1958.
15. WISE, R. I.: Principles of management of staphylococci infections, *J.A.M.A.* 166:1178, 1958.
16. ROMANSKY, M. J.: Personal communication.
17. HAIGHT, T. H., AND FINLAND, M.: The antibacterial action of erythromycin, *Proc. Soc. Exper. Biol. & Med.* 8:175, 1952.
18. BIGGER, J. W.: The treatment of staphylococcal infections with penicillin by intermittent sterilization, *Lancet* 2:497-500, 1944.
19. McDERMOTT, W.: Microbial persistence. *Yale J. Biol. & Med.* 30:257, 1958.

# Studies on Synergists for Antimicrobial Agents

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The phenomenon of synergism and/or antagonism between two antimicrobial agents has been discussed by many workers.<sup>1-3</sup>

This study was undertaken with the assumption that an organism subjected to a subinhibitory concentration of an antibiotic may be altered in its physiology and biochemical activity, possibly in a manner that would make it more susceptible (or resistant) to certain agents that have no detectable inhibitory effect when used alone. Such a study might attain two main goals: synergists may be found that will permit the clinical use of lower concentrations of the more toxic antibiotics, and the developing patterns of interaction may give us greater insight into the modes of action of these antibiotics.

We are reporting here a series of such studies using amino acids, their anti-metabolites, and related compounds.

## MATERIALS AND METHODS

The basic procedure utilized was the strip-gradient plate technique.<sup>4,5</sup> Gradient plates were prepared with medium no. 3 of Grove and Randall,<sup>6</sup> to which agar was added. For the more diffusible antibacterial substances, routine laboratory agar-agar was used at 1.5 per cent concentration. For the large molecule antibiotics, 0.75 per cent Ionagar\* no. 2 agar was used, since it permits better diffusion than conventional agars.<sup>7</sup> Each layer of the gradient contained 12.5 ml. of medium, with the antibiotic incorporated in the upper layer. These plates were overlaid with 3.5 ml. of seeded agar.

The test organisms used in these studies were *Staphylococcus aureus* 209 (ATCC 6538P) and *Sarcina lutea* (ATCC 9341). Plates were incubated for 18 hours at 35 C. before being read.

The antibiotic solutions were prepared in accordance with the instructions of Grove and Randall.<sup>6</sup> The antibiotics were dissolved in the upper layers of the gradient plates at the following concentrations: *S. lutea*: penicillin, 0.03 unit; chloramphenicol, 0.30  $\mu$ g. *Staphylococcus*: penicillin, 0.04 unit; chloramphenicol, 2.0  $\mu$ g.; erythromycin, 0.25  $\mu$ g.; bacitracin, 60  $\mu$ g.; polymyxin B, 150  $\mu$ g.; ristocetin, 5  $\mu$ g.; oxytetracycline, 0.20  $\mu$ g.; chlortetracycline, 0.10  $\mu$ g.; tetracycline, 0.10  $\mu$ g.

All 75 agents were run against the test organisms on gradient plates without antibiotics added.

Synergism or antagonism is recorded as a fraction in which the numerator (h) is the distance in mm. representing the limiting concentrations of the agent in the strip, which is active for any particular concentration of the antibiotic, and in which the denominator (v) is the distance in mm. representing the limiting concentrations of the antibiotic, which is active for any particular concentration of the agent in the strip (see figure 1). Where both synergism and antagonism occurred on the same plate, these are so recorded with no attempt at measurement. Plates on which the

\* Trade name of Oxo, Ltd., London. This agar can be procured from Consolidated Laboratories, Inc., Chicago Heights, Ill.

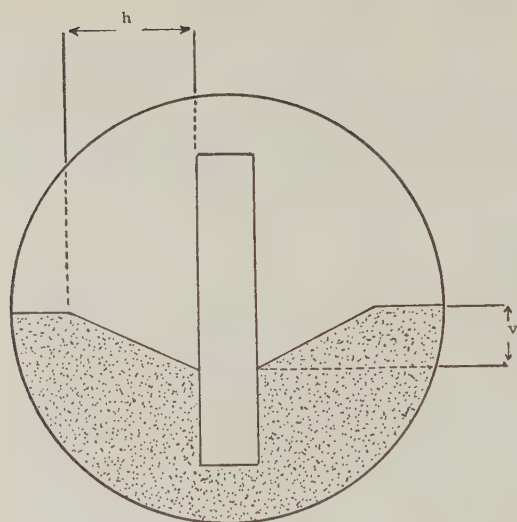


FIG. 1. Shown is the key to evaluation of synergism or antagonism on strip-gradient plate. The plate shows synergism. Bacterial growth is on the bottom. Tables I and III summarize data using the fraction:  $h/v$ .

zone of inhibition was less than 5 mm. are recorded as showing a trace of activity. All tests were run in duplicate.

## RESULTS

Table I summarizes the data for interaction between the agents tested and penicillin and chloramphenicol, with *S. lutea* as the test organism; it also presents the interaction between these agents and antibiotics, as well as oxytetracycline, tetracycline, and chlortetracycline, with *Staphylococcus* as the test organism.

Table II summarizes the comparative reactivity of the 75 agents. It is clear that chloramphenicol is much less affected in its activity than penicillin.

Table III summarizes the data for the interaction between the 75 agents tested and four antibiotics: erythromycin, bacitracin, polymyxin B, and ristocetin, using *Staphylococcus* as the test organism.

Table IV summarizes the data for the nine antibiotics tested against these agents with *Staphylococcus* as the test organism. It will be noted that the pattern of reactivity noted with *S. lutea* is consistent here in that the activity of chloramphenicol is again least affected by these agents. It is quite striking that all but four of the agents exhibited some effect on the activity of chlortetracycline and that tetracycline was nearly as much affected, while 38 agents showed no effect on the activity of oxytetracycline.

Figures 2 to 7 are representative of the various patterns of synergism and/or antagonism found with the agents and antibiotics here tested.

## DISCUSSION

We might expect that any two antibacterial substances that shared some facet of their mechanism of action might show some consistency in their susceptibility to antagonism or synergism by the agents here studied. No such relationships were apparent with any degree of regularity. It is possible that the use of agents in such a test might be too discriminating to point out similarities in activity unless very closely allied antibacterial agents were studied. However, when the tetracyclines are noted, only two agents showed consistent results: kinetin and furfuryl kinetin. Since these agents are adenine derivatives, it would seem that our attention here should

TABLE I

Strip-Gradient Plate Results\*

Agent on strip, 1000 $\mu\text{g.}/\text{strip}$	<i>S. lutea</i>				<i>Staph. aureus</i> 209				
	No antibiotic	Penicillin	Chloram- phenicol	Oxytetra- cycline	Tetra- cycline	Chlortet- racycline	Penicillin	Chloram- phenicol	No antibiotic
D-Leucine	Neg.	S. 30/5	Neg.	Neg.	Tr. S. and A.	S. 5/30	Tr. S.	Neg.	Neg.
L-Leucine	Neg.	Tr. S.	S. 25/5	Neg.	S. 5/10	S. 10/25	Tr. S.	Neg.	Tr. Inh.
D-Methionine	Neg.	Tr. S.	Neg.	Neg.	Tr. S. and A.	S. 15/30	Neg.	Neg.	Neg.
L-Methionine	Neg.	Neg.	Neg.	Tr. A.	Tr. S. and A.	S. 15/30	Neg.	Neg.	Neg.
DL-Ethionine	Neg.	Neg.	Neg.	Neg.	Tr. S. and A.	S. 10/25	Neg.	Neg.	Neg.
DL-Norleucine	Neg.	Tr. A.	Neg.	Neg.	Tr. A.	Tr. S. and A.	Neg.	Neg.	Neg.
Methionine sulfoxide	Neg.	Tr. S.	Neg.	Tr. A.	Tr. S. and A.	S. 15/20	Tr. S.	Tr. S. and A.	Neg.
DL-Methionine sulfone	Neg.	S. 30/5	Neg.	Tr. S.	Tr. S. and A.	S. 10/20	S. 10/10	Neg.	Neg.
D-Serine	Neg.	S. 7/5	S. 5/10	Neg.	Tr. S. and A.	S. 10/20	S. 7/3	Neg.	Neg.
L-Serine	Neg.	Neg.	Neg.	Tr. S.	Tr. S.	S. 15/30	Neg.	Neg.	Neg.
$\beta$ -2-Thienylserine	Neg.	Neg.	Neg.	Tr. A.	Tr. S.	S. 5/15	Neg.	Neg.	Neg.
$\alpha$ -Methylserine	Neg.	Tr. S.	Neg.	S. 30/5	A. 20/15	S. 10/25	S. 40/10	Neg.	Neg.
D-Alanine	Tr. Stim.	S. 30/5	Tr. S.	Neg.	Neg.	S. 5/25	A. 5/10	Tr. S.	Neg.
L-Alanine	Tr. Stim.	Neg.	Tr. S.	Neg.	Tr. A.	Tr. S. and A.	Tr. S. and A.	Tr. S.	Neg.
L-Asparagine	Neg.	S. 5/10	Neg.	Neg.	S. 15/25	S. 12/25	Neg.	Neg.	Neg.
Glycine	Neg.	Neg.	Neg.	Neg.	Tr. S. and A.	S. 5/20	Tr. S.	Neg.	Neg.
D-Phenylalanine	Neg.	Neg.	Neg.	Neg.	Tr. A.	S. 10/10	Neg.	Neg.	Neg.
L-Phenylalanine	Neg.	Neg.	Neg.	Neg.	Tr. S.	S. 10/40	Neg.	Neg.	Neg.
$\beta$ -(2-Thienyl) DL-alanine	Neg.	Neg.	Neg.	Tr. A.	Neg.	S. 7/20	Neg.	S. 5/5	Neg.
DL- $\beta$ -Phenyl-lactic acid	Neg.	Neg.	Neg.	Tr. A.	Tr. A.	S. 10/20	Neg.	Neg.	Neg.
D-Tryptophane	Neg.	S. 30/5	Neg.	Neg.	S. 10/10	S. 7/40	Tr. S.	Neg.	Neg.
L-Tryptophane	Neg.	Neg.	Neg.	Tr. A.	Tr. S. and A.	A. 5/20	Tr. A.	Neg.	Neg.
Indole	Neg.	Neg.	Neg.	Tr. S.	Tr. A.	Tr. S. and A.	Neg.	Neg.	Neg.
Skatole	Neg.	Neg.	Neg.	S. 10/10	Tr. S.	Tr. S.	Neg.	Neg.	Neg.
L-Tyrosine	Neg.	Neg.	Neg.	Tr. A.	Tr. A.	A. 3/10	Tr. A.	S. 5/35	Neg.
L-3-Aminotyrosine	Tr. Stim.	Neg.	Neg.	Tr. A.	Tr. S. and A.	S. 5/20	Neg.	Neg.	Neg.
D-Cystine	Neg.	Tr. S.	Neg.	Tr. A.	Tr. A.	Neg.	Neg.	S. 5/7	Neg.
L-Cystine	Neg.	Neg.	Neg.	Tr. A.	Neg.	A. 5/10	Neg.	Neg.	Neg.
Allyl glycine	Neg.	Tr. S.	Neg.	Neg.	Tr. A. and S.	S. 25/30	Neg.	Neg.	Neg.

Table I Continued on Page 858

TABLE I (Continued)

Strip-Gradient Plate Results\*

Agent on strip, 1000 $\mu$ g./strip	<i>S. lutea</i>			<i>Staph. aureus</i> 209					
	No antibiotic	Penicillin	Chloram- phenicol	Oxytetra- cycline	Tetra- cycline	Chlortet- racycline	Penicillin	Chloram- phenicol	No antibiotic
Thiazolidine-4-carboxylic acid	Neg.	S. 30/10	Neg.	S. 20/10	Neg.	S. 5/30	S. 7/5	Neg.	Neg.
$\beta$ -Methyl-DL-cystine (isomer A) <sup>†</sup>	Neg.	S. 30/10	Neg.	Tr. S.	S. 10/10	Tr. S.	Tr. S.	Tr. S.	Tr. Stim.
$\beta$ -Methyl-DL-cystine (isomer B) <sup>†</sup>	Neg.	Tr. S.	Neg.	Tr. S.	S. 10/10	Tr. S.	Neg.	Neg.	Tr. Stim.
N-Methyl-L-cystine <sup>†</sup>	Tr. Stim.	Neg.	Neg.	S. 20/10	S. 15/10	Tr. S.	Tr. S.	Neg.	Tr. Stim.
$\alpha$ -Methyl-DL-cystine <sup>†</sup>	Tr. Stim.	Neg.	Neg.	Tr. S.	S. 30/10	S. 15/10	Tr. S.	Neg.	Tr. Stim.
$\beta$ -Mercaptopropionic acid	Tr. Inh.	S. 6/10	Neg.	Neg.	A. 5/5	S. 10/25	S. 5/10	Neg.	Neg.
D-Aspartic acid	Neg.	Tr. S.	Neg.	Neg.	S. 8/20	S. 15/30	A. 10/10	Neg.	Neg.
L-Aspartic acid	Tr. Inh.	Tr. S.	S. 5/4	Neg.	S. 12/30	S. 10/30	Tr. A.	Neg.	Neg.
D-Glutamic acid	Tr. Inh.	S. 10/10	Tr. S.	Tr. A.	A. 5/5	S. 10/40	Tr. S. and A.	Neg.	Tr. Inh.
L-Glutamic acid	Tr. Inh.	Neg.	Neg.	Neg.	Tr. S.	S. 15/25	A. 10/10	Neg.	Neg.
L-Proline	Neg.	Tr. A.	Neg.	Neg.	Tr. S. and A.	S. 10/25	Tr. A. and S.	Neg.	Neg.
D-Hydroxyproline	Neg.	Tr. S.	Neg.	Tr. A.	Tr. S. and A.	S. 10/25	A. 7/5	Neg.	Neg.
L-Hydroxyproline	Neg.	Tr. S.	Neg.	Tr. A.	S. 12/15	S. 10/20	Tr. A.	Neg.	Neg.
L-Citrulline	Neg.	Tr. A.	Neg.	Tr. A.	Tr. A.	S. 8/30	A. 7/5	Neg.	Neg.
L-Lysine	Tr. Inh.	Tr. S. and A.	Neg.	Neg.	Tr. A.	S. 8/40	Tr. S. and A.	Neg.	Neg.
D-Histidine	Neg.	Tr. S.	Neg.	Neg.	Tr. S. and A.	Tr. S. and A.	Tr. A.	Neg.	Neg.
L-Histidine	Neg.	A. 35/5	Neg.	Tr. A.	Tr. S. and A.	Tr. S.	A. 7/10	Neg.	Neg.
D-Isoleucine	Neg.	Neg.	Neg.	Neg.	Tr. S. and A.	Tr. A.	Tr. A.	Neg.	Neg.
L-Isoleucine	Neg.	Tr. A.	Neg.	Neg.	Tr. A.	Neg.	A. 7/15	Neg.	Neg.
D-Threonine	Neg.	Tr. S.	Neg.	Neg.	Tr. A.	S. 7/45	Tr. A.	Neg.	Neg.
L-Threonine	Tr. Inh.	Neg.	Neg.	Neg.	Tr. S.	A. 5/10	Tr. A.	Neg.	Neg.
D-Valine	Neg.	Tr. S.	Neg.	Tr. A.	Tr. S.	Tr. A.	Tr. A.	Neg.	Neg.
L-Valine	Neg.	Tr. S. and A.	Neg.	Tr. A.	Tr. S.	S. 5/30	A. 7/5	Neg.	Neg.
DL-Norvaline	Tr. Stim.	Neg.	Neg.	Neg.	Tr. A.	Tr. S.	Neg.	Tr. S.	Tr. Inh.
L-Arginine	Tr. Inh.	Neg.	Neg.	Neg.	Tr. A.	Tr. A.	Tr. A.	Neg.	Neg.
Cannavanine sulfate	Neg.	Neg.	Neg.	Neg.	Tr. S.	S. 10/25	Neg.	Neg.	Neg.

Table I Continued on Page 859

TABLE I (Continued)

Strip-Gradient Plate Results\*

Agent on strip, 1000 $\mu\text{g.}/\text{strip}$	<i>S. lutea</i>			<i>Staph. aureus</i> 209					
	No antibiotic	Penicillin	Chloram- phenicol	Oxytetra- cycline	Tetra- cycline	Chlortet- racycline	Penicillin	Chloram- phenicol	No antibiotic
Colchicine	Tr. Inh.	Tr. S.	Neg.	Neg.	Tr. S. and A.	S. 3/25	Neg.	Neg.	Neg.
6-Mercaptopurine	Neg.	A. 30/10	Tr. S.	Tr. S.	Tr. A and S.	Tr. A. and S.	Neg.	Neg.	Neg.
Kinetin †	Neg.	Tr. S.	Neg.	S. 15/10	S. 10/10	S. 10/10	S. 10/15	Tr. S.	Tr. Inh.
Furfuryl kinetin †	Neg.	Tr. S.	Neg.	S. 10/10	S. 15/10	S. 10/10	S. 5/15	Neg.	Tr. Inh.
p-Amino benzoic acid	Neg.	Tr. S.	Neg.	S. 25/15	Tr. S.	S. 25/10	Tr. S.	Neg.	Neg.
Pyridine-3-sulfonic acid ‡	Neg.	S. 15/10	S. 10/50	Tr. S.	S. 15/25	S. 20/10	Tr. S.	A. 7/5	Tr. Stim.
D-Penicillamine	Neg.	S. 5/5	S. 5/30	Tr. S.	Tr. S. and A.	Tr. S. and A.	Tr. S.	Tr. S. and A.	Neg.
L-Penicillamine	Neg.	Neg.	Neg.	Neg.	S. 10/20	S. 15/20	Neg.	Neg.	Neg.
Mercaptoacetic acid	Tr. Stim.	Tr. A.	Neg.	Tr. A.	Tr. S. and A.	Tr. S.	Tr. S.	Tr. S.	Neg.
Indole-3-acetic acid	Neg.	S. 5/5	Neg.	Neg.	A. 10/5	S. 20/10	Neg.	Neg.	Neg.
2,4,6-Trichlorophenoxyacetic acid	Neg.	S. 10/10	Neg.	Neg.	Tr. A. and S.	Tr. A. and S.	S. 10/25	S. 12/5	Neg.
Naphthalene acetic acid	Neg.	S. 15/10	Neg.	Neg.	A. 15/15	S. 10/15	A. 10/5	Neg.	Neg.
$\alpha,\alpha$ -Dichloropropionic acid	Neg.	Tr. S.	Neg.	Neg.	S. 5/10	S. 30/30	Neg.	Neg.	Neg.
$\beta$ -Isothiouridopropionic acid	Neg.	Neg.	Neg.	Tr. S.	Tr. S.	Tr. S.	Neg.	Neg.	Neg.
$\alpha$ -Phenoxypropionic acid	Neg.	A. 30/10	Neg.	Neg.	Tr. A. and S.	S. 10/10	Neg.	Neg.	Neg.
Indole-3-propionic acid	Neg.	Tr. A.	Tr. S.	S. 15/15	Tr. S.	Tr. S.	Neg.	Tr. S.	Tr. Inh.
$\alpha$ (2,4-Dichlorophenoxy) propionic acid	Neg.	Tr. S.	Neg.	Tr. A. and S.	Neg.	Tr. S.	Neg.	Tr. S.	Neg.
$\alpha$ (2,4,5-Trichlorophenoxy) propionic acid	Neg.	Tr. S.	Neg.	Tr. S.	Tr. S.	Tr. S.	S. 12/20	Tr. S.	Tr. Inh.
Sodium acetate	Neg.	Tr. A.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Sodium propionate	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

\* Neg. = no activity; Tr. = trace of activity; Inh. = inhibition of growth; Stim. = stimulation of growth; S. = synergism; A. = antagonism.

† Five hundred  $\mu\text{g.}/\text{strip}$ .‡ One hundred  $\mu\text{g.}/\text{strip}$ .

TABLE II  
*Comparative Reactivity of Two Antibacterial Agents with  
the 75 Compounds Studied Using S. lutea (Compiled from Table I)*

Reaction	Penicillin	Chloramphenicol
Frank synergism	15	6
Trace of synergism	21	5
Trace of synergism and antagonism	2	0
No effect	27	64
Frank antagonism	3	0
Trace of antagonism	7	0
Total activity	48	11

be focused on nucleic acid activity. In his review on oxytetracycline, Musselman<sup>8</sup> noted that the antibiotic interfered with metal ion utilization and protein synthesis. Our data would seem to suggest that the interference with protein synthesis is at a level higher than amino acid metabolism. Lepper,<sup>9</sup> in his review of chlortetracycline, made similar observations for that substance, and our data also confirm his contention that the chlortetracycline activity is influenced by a much larger variety of environmental factors and agents than is any other antibacterial substance studied to date. Dowling,<sup>10</sup> in his review of tetracycline, made similar observations of the mode of action of that substance.

It was of considerable interest that 6 of the 14 more active synergists were agents that have reported plant hormone activity. All of the plant hormones included in this study were active. We are not in a position as yet to comment on the significance of this observation, but it does pose a point worthy of continued study both from the standpoint of their activity as antibacterial synergists and from the standpoint of their usefulness as a tool in elucidating a complete picture of the mode of action of antibacterial substances.

Numerous examples of synergism with amino acids were found and are shown

TABLE III  
*Strip-Gradient Plate Results with Staph. aureus 209\**

Agent on strip, 1000 µg./strip	Antibacterial agent in gradient				
	None	Erythro- mycin	Baci- tracin	Polymyxin B	Risto- cetin
D-Leucine	Neg.	Tr. S.	Neg.	Neg.	Tr. A. and S.
L-Leucine	Tr. Inh.	Neg.	Tr. S.	Neg.	S. 12/27
D-Methionine	Neg.	Tr. S.	Tr. A.	Neg.	Tr. S.
L-Methionine	Neg.	Neg.	Neg.	Neg.	Tr. S.
DL-Ethionine	Neg.	Neg.	Tr. S.	S. 30/10	S. 25/14
DL-Norleucine	Neg.	Tr. S.	Neg.	Neg.	Tr. A.
Methionine sulfoxide	Neg.	Tr. S.	Tr. A.	Neg.	Tr. S.
DL-Methionine sulfone	Neg.	S. 30/15	Tr. S.	Tr. S. and A.	Tr. S.
D-Serine	Neg.	Tr. S.	Neg.	S. 6/8	Tr. S. and A.
L-Serine	Neg.	Neg.	Tr. A.	S. 30/20	Tr. S.
beta-2-Thienylserine	Neg.	Tr. S.	Tr. A.	S. 30/15	Neg.
alpha-Methylserine	Neg.	S. 15/10	Neg.	S. 27/5	S. 20/10
D-Alanine	Neg.	Tr. S.	Neg.	Neg.	Neg.
L-Alanine	Neg.	Neg.	Neg.	Neg.	Neg.
L-Asparagine	Neg.	Tr. A.	Tr. S.	Neg.	Neg.
Glycine	Neg.	Tr. S.	Tr. S.	Neg.	Tr. A.
D-Phenylalanine	Neg.	Tr. A.	Tr. S.	Neg.	Tr. A.
L-Phenylalanine	Neg.	Tr. A.	Neg.	Neg.	Neg.

*Table III Continued on Page 861*

TABLE III (Continued)  
Strip-Gradient Plate Results with *Staph. aureus* 209\*

Agent on strip, 1000 $\mu$ g./strip	Antibacterial agent in gradient				
	None	Erythro- mycin	Baci- tracin	Polymyxin B	Risto- cetin
beta(2-Thienyl)DL-alanine	Neg.	Tr. S.	Tr. S.	Tr. A.	Tr. A.
DL-Beta-Phenyllactic acid	Neg.	Tr. A.	Neg.	Neg.	Tr. A. and S.
D-Tryptophane	Neg.	Tr. A.	Neg.	Neg.	Tr. S.
L-Tryptophane	Neg.	Tr. S.	Tr. A.	Tr. S.	Neg.
Indole	Neg.	S. 12/15	Tr. S.	Tr. S.	Tr. S.
Skatole	Neg.	Neg.	Tr. S.	Neg.	S. 30/10
L-Tyrosine	Neg.	Tr. S.	Neg.	Tr. S.	Tr. S.
L-3-Aminotyrosine	Neg.	Neg.	A. 12/15	Neg.	Tr. A.
D-Cystine	Neg.	Tr. S.	Tr. A.	Neg.	Tr. S.
L-Cystine	Neg.	S. 10/10	Neg.	Tr. S.	Tr. S.
Allyl glycine	Neg.	Neg.	Neg.	Neg.	Neg.
Thiazolidine-4-carboxylic acid	Neg.	Neg.	Tr. S.	Neg.	Tr. S.
beta-Methyl-DL-cystine (isomer A)	Tr. Stim.†	Tr. A.†	Tr. S.†	Neg.†	Tr. A.†
beta-Methyl-DL-cystine (isomer B)	Tr. Stim.†	Neg.†	Tr. S.†	Neg.†	Tr. S.†
N-Methyl-L-cystine	Tr. Stim.†	Neg.†	Tr. S.†	Neg.†	Neg.†
alpha-Methyl-DL-cystine	Tr. Stim.†	Neg.†	S. 15/15†	Neg.†	Tr. A.†
beta-Mercaptopropionic acid	Neg.	A. 40/15	S. 10/15	Tr. A.	Tr. A.
D-Aspartic acid	Neg.	Tr. A.	Tr. A.	Neg.	Tr. S. and A.
L-Aspartic acid	Neg.	Tr. A.	Tr. S.	Neg.	Neg.
D-Glutamic acid	Tr. Inh.	A. 7/20	S. 15/30	S. 13/40	Tr. S. and A.
L-Glutamic acid	Neg.	Tr. A.	S. 12/25	Tr. S. and A.	Tr. S.
L-Proline	Neg.	Tr. S.	A. 10/10	Neg.	Neg.
D-Hydroxyproline	Neg.	Tr. A.	Tr. S.	Neg.	Neg.
L-Hydroxyproline	Neg.	Neg.	Neg.	Neg.	Neg.
L-Citrulline	Neg.	Tr. A.	Tr. A.	Neg.	Tr. S.
L-Lysine	Neg.	Tr. S.	Neg.	Neg.	Neg.
D-Histidine	Neg.	Neg.	Tr. A.	Neg.	Neg.
L-Histidine	Neg.	Neg.	A. 10/10	Neg.	Neg.
D-Isoleucine	Neg.	Neg.	Neg.	Neg.	Neg.
L-Isoleucine	Neg.	Tr. S.	Tr. A.	Neg.	Neg.
D-Threonine	Neg.	Tr. A.	Tr. A.	Neg.	Tr. S. and A.
L-Threonine	Neg.	Neg.	Tr. A.	Neg.	Tr. S. and A.
D-Valine	Neg.	Tr. A.	Tr. A.	Neg.	Tr. S. and A.
L-Valine	Neg.	Tr. A.	S. 25/3	Neg.	Neg.
DL-Norvaline	Tr. Inh.	Neg.	Tr. A.	Neg.	Neg.
L-Argenine	Neg.	Tr. A.	A. 6/10	Neg.	Neg.
Cannavanine sulfate	Neg.	Tr. A.	Tr. S.	Tr. A.	Tr. A.
Colchicine	Neg.	Neg.	S. 25/5	Neg.	Tr. S.
6-Mercaptopurine	Neg.	Tr. A.	Neg.	Neg.	A. 35/10
Kinetin	Tr. Inh.†	Neg.†	Neg.†	Tr. A.†	A. 10/10†
Furfuryl kinetin	Tr. Inh.†	Neg.†	S. 5/15†	Neg.†	Tr. A.†
p-Aminobenzoic acid	Neg.	Tr. S.	S. 3/25	Neg.	Tr. S.
Pyridine-3-sulfonic acid	Tr. Stim.†	Tr. S.	S. 10/15	S. 11/52	Tr. S.
D-Penicillamine	Neg.	Tr. A.	S. 15/25	S. 12/15	Tr. S. and A.
L-Penicillamine	Neg.	Neg.	S. 10/10	Neg.	S. 25/10
Mercaptoacetic acid	Neg.	Tr. A.	Tr. A.	Tr. A.	A. 10/10
Indole-3-acetic acid	Neg.	S. 10/5	Tr. A.	Neg.	A. 7/5
2,4,6-Trichlorophenoxyacetic acid	Neg.	S. 25/30	S. 22/10	S. 20/35	S. 25/15
Naphthalene acetic acid	Neg.	S. 7/10	Neg.	Tr. S.	Tr. A.
alpha, alpha'-Dichloropropionic acid	Neg.	A. 15/15	S. 12/20	S. 7/20	S. 30/10
beta-Isothiouriedopropionic acid	Neg.	Neg.	Tr. S.	Tr. A.	Neg.
alpha-Phenoxypropionic acid	Neg.	S. 20/10	Neg.	Neg.	Tr. A. and S.
Indole-3-propionic acid	Tr. Inh.	Tr. S.	S. 5/10	Tr. A.	Tr. A.
alpha(2,4-Dichlorophenoxy) propionic acid	Neg.	S. 10/15	Tr. S.	S. 5/35	Tr. S.
alpha(2,4,5-Trichlorophenoxy) propionic acid	Tr. Inh.	S. 10/10	S. 10/20	S. 10/60	S. 25/15
Sodium acetate	Neg.	Neg.	Neg.	Neg.	Neg.
Sodium propionate	Neg.	Neg.	Neg.	Neg.	Tr. A.

\* For code, see table I.

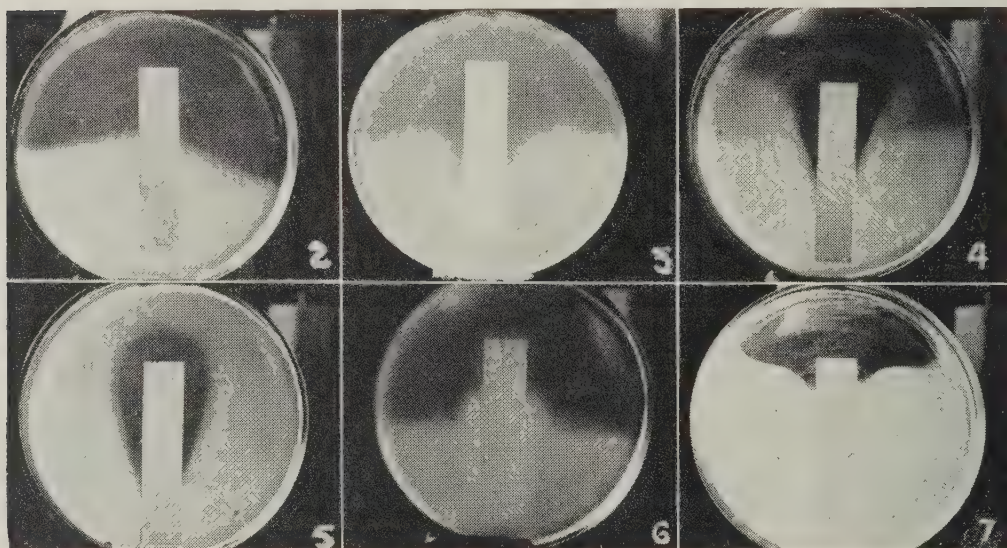
† Five hundred  $\mu$ g. of agent used per strip, due to short supply of these rare compounds.

TABLE IV  
Comparative Reactivity of 9 Antibiotics with the 75  
Compounds Studied Using *Staph. aureus* 209 (Compiled from Tables I and III)

Reaction	Peni- cillin	Chlor- amphen- icol	Eryth- ro- mycin	Oxy- tetra- cycline	Tetra- cycline	Chlor- tetra- cycline	Baci- tracin	Poly- myxin B	Risto- cetin
Frank synergism	9	4	10	8	15	46	15	12	8
Trace of synergism	12	9	18	11	12	11	18	5	18
No effect	31	59	25	38	7	4	22	49	22
Trace of antagonism and synergism	4	2	0	1	22	7	0	2	10
Trace of antagonism	10	0	19	17	14	3	16	7	13
Frank antagonism	9	1	3	0	5	4	4	0	4
Total activity	44	16	50	37	68	71	53	26	53

in the tables. The reason that a natural amino acid should show synergism with an antibacterial substance is not clear unless the antibiotic disturbs the amino acid metabolism in a way that makes possible competitive inhibition with related natural amino acids. Such competitive inhibition with amino acids has been reported.<sup>11</sup> Since none of the amino acids used here showed any marked inhibition when used alone, the possible explanation just presented would have to await a more precise understanding of the mode of action of the antibiotics.

Sodium acetate and propionate were included to exclude the possibility that ace-



The high concentration of the antibiotics are in the upper portions of all the plates. See text for details of concentrations of antibiotics and agents impregnated in strips.

FIG. 2. *S. lutea* is exposed to 6-mercaptopurine and penicillin. Antagonism apparent over a wide range of agent in strip but over a narrow range of penicillin.

FIG. 3. *S. aureus* is exposed to 2,4,6-trichlorophenoxyacetic acid and penicillin.

FIG. 4. *S. aureus* is exposed to pyridine-3-sulfonic acid and bacitracin. Note that there are two zones of inhibition showing synergism.

FIG. 5. *S. aureus* is exposed to pyridine-3-sulfonic acid and polymyxin B. The synergism here is very good. Note that antibiotic itself is not inhibitory even at the highest concentration.

FIG. 6. *S. aureus* is exposed to L-glutamic acid and penicillin. Antagonism over a very narrow range of antibiotic and agent in strip.

FIG. 7. *S. aureus* is exposed to 2,4,6-trichlorophenoxyacetic acid and ristocetin. Note that there are two levels of synergism interrupted by a zone of inactivity.

tate or propionate ions were responsible for reactions with agents having these radicals.

Where the D and L isomers of an agent were studied, it was clear that in most instances one of the isomers showed more pronounced activity than the others. However, we could not conclude that one isomer was in general more consistently active than the other. As examples, although D-methionine, D-penicillamine, and D-hydroxyproline were generally more active than their L isomers, D- and L-glutamic acid were equally effective with polymyxin B, ristocetin, and bacitracin. L-Histidine showed much more antagonism for penicillin than the D isomer; this is also the case with this agent when used with bacitracin. However, any indication of a pattern in this respect breaks down completely with the tetracyclines.

It is most significant that a few of the compounds here studied showed consistent synergistic activity with a variety of antibiotics and organisms studied. For example, 2, 4, 6-trichlorophenoxy acetic acid showed frank synergism with penicillin against *S. lutea*, with penicillin, chloramphenicol, erythromycin, bacitracin, polymyxin B, and ristocetin against *Staph. aureus*, and with isoniazid and streptomycin against *Mycobacterium tuberculosis* (this work to be reported elsewhere). Such similar broad activity has also been found to occur with D-penicillamine.

The primary significance of the study here reported is the possible use of antibiotics, whose use at certain levels is precluded at present because of their toxicity, at lowered dosage levels, which will be bactericidal or bacteriostatic when used concomitantly with a nontoxic synergist such as described here.

#### SUMMARY

Using the strip-gradient plate technique, it was demonstrated that compounds having little or no antimicrobial activity when used alone acted as synergists when mixed with antibiotics. The antibacterial substances studied were penicillin, chloramphenicol, erythromycin, bacitracin, polymyxin B, ristocetin, oxytetracycline, chlor-tetracycline, and tetracycline. The most active synergists of the 75 agents studied were: D-penicillamine, 2,4,6-trichlorophenoxy acetic acid, indole-3-propionic acid,  $\alpha$ (2,4-dichlorophenoxy)propionic acid,  $\alpha$ (2,4,5-trichlorophenoxy)propionic acid, and pyridine-3-sulfonic acid. Over-all observation of patterns of activity may help to elucidate the mode of action of the various antibacterial substances studied. It is proposed that such synergism with compounds might make feasible the increased therapeutic range of the more toxic antibiotics.

#### ACKNOWLEDGMENTS

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## BIBLIOGRAPHY

1. GUNNISON, J. B.; JAWETZ, E., AND COLEMAN, V. R.: The effect of combinations of antibiotics on enterococci *in vitro*, J. Lab. & Clin. Med. 36:900-911, 1950.
2. GUNNISON, J. B.; COLEMAN, V. R., AND JAWETZ, E.: Interference of Aureomycin and of Terramycin with action of penicillin *in vitro*, Proc. Soc. Exper. Biol. & Med. 75:549-552, 1950.
3. GUNNISON, J. B.; SPECK, R. S.; JAWETZ, E., AND BRUFF, J. A.: Studies on antibiotic synergism and antagonism: the effect of sulfadiazine on the action of penicillin *in vitro* and *in vivo*, Antib. & Chemo. 1:259-266, 1951.
4. STREITFIELD, M. M., AND GUADANO, J. J.: Replica strip-gradient plate, a method for testing bacteriostatic or bactericidal action of antibiotics paired in various concentration ratios, Bact. Proc., 1956, p. 30.
5. STREITFIELD, M. M.: The replica strip-gradient plate technique for determination of synergism or antagonism of antibiotics paired in various concentration ratios: a bacteriostatic and bactericidal assay. In: Antibiotics Annual 1956-1957, New York, Medical Encyclopedia, Inc., 1957, pp. 906-917.
6. GROVE, D. C., AND RANDALL, W. A.: Assay Methods of Antibiotics: A Laboratory Manual, New York, Medical Encyclopedia, Inc., 1955.
7. BECHTLE, R. M., AND SCHERR, G. H.: A new agar for *in vitro* antimicrobial sensitivity testing, Antib. & Chemo. 8:599, 1958.
8. MUSSELMAN, M. M.: Terramycin (Oxytetracycline), New York, Medical Encyclopedia, Inc., 1956.
9. LEPPER, M. H.: Aureomycin (Chlortetracycline), New York, Medical Encyclopedia, Inc., 1956.
10. DOWLING, H. F.: Tetracycline, New York, Medical Encyclopedia, Inc., 1955.
11. COHEN, G. N., AND RICKENBERG, H. V.: Concentration spécifique reversible des amino acids chez *Escherichia coli*, Ann. Inst. Pasteur 91:693, 1956.

# The Growth Rates and Adaptive Enzyme Activities of Chloramphenicol- and Oxytetracycline-Resistant *Escherichia coli*

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The present report presents selected data on the growth rates and the adaptive enzyme activities of stocks of *Escherichia coli* resistant to chloramphenicol and to oxytetracycline. Although it is evident that the actions of these two antibiotics are generally similar, some interesting and possibly important quantitative differences are apparent.

## EXPERIMENTAL

The cultures used were all derived from a single auxotrophic mutant of the K 12 strain of *E. coli*; this parental form will be designated later as the reference stock. Since this latter stock and all the variants derived from it required biotin and phenylalanine, these substances were added to all media when growth was desired. Culture 1 was selected for increased resistance to oxytetracycline by serial passages of the reference stock through increasing amounts of that antibiotic contained in brain-heart broth. Cultures 2 to 6, inclusive, were selected by similar passages in chloramphenicol-containing brain-heart broth.

The media used for the quantitative assay of the degree of resistance contained potassium monohydrogen phosphate, 7 Gm.; potassium dihydrogen phosphate, 2 Gm.; ammonium sulfate, 1 Gm.; sodium citrate, 3 Gm.; dl-phenylalanine, 100 mg.; glucose, 3 Gm.; hydrated magnesium sulfate, 100 mg.; biotin, 5  $\mu$ g./liter. In the experiments on growth stimulation by an antibiotic the medium contained salts, as indicated, supplemented with glucose, 2 Gm.; dl-phenylalanine, 40 mg.; and biotin, 5  $\mu$ g./liter. Certain tubes contained, additionally, yeast extract (Difco) or amino acids (N-Z-Case peptone) or both, as will be specified. Chloramphenicol was kindly supplied by the Parke, Davis & Co. as the crystalline form. The oxytetracycline used was the hydrochloride of Chas. Pfizer and Co., 1.0 Gm. of which is stated to be equivalent to 0.88 Gm. of oxytetracycline.

For the assay of resistance the tubes were incubated at 37 C., with aeration by shaking. The amounts of growth were estimated in optical density units as read on a Coleman Jr. spectrophotometer. The degree of resistance was determined from the amounts of antibiotic that reduced the growths to 50 per cent of that of the drug-free control, as estimated from plots on logarithmic-normal graph paper<sup>1</sup> (table I).

*Growth Stimulation by Chloramphenicol and by Oxytetracycline.* As we have noted, the resistance titrations were made in a fortified synthetic medium. Their course was entirely normal, i.e., once the threshold of antibiotic activity had been reached the bacterial growth declined regularly with increasing drug concentrations.<sup>1</sup> When trial assays were made in brain-heart broth, however, the growth of

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\* Rockefeller Foundation Fellow, on leave from the National Institute of Health, Tokyo, Japan.

TABLE I  
Absolute and Relative Antibiotic Sensitivities of *Escherichia coli* Stocks Utilized

Culture	Chloramphenicol		Oxytetracycline	
	$\mu\text{g.}/\text{ml.}$ for 50% inhibition	Ratio to reference culture	$\mu\text{g.}/\text{ml.}$ for 50% inhibition	Ratio to reference culture
Parent, reference	0.28	1	0.042	1
Resistant 1	5.3	19	0.34	8.1
Resistant 2	2.0	7.1	0.45	12
Resistant 3	6.7	24	0.18	4.3
Resistant 4	5.0	18	0.13	3.1
Resistant 5	3.3	12	0.35	8.2
Resistant 6	24	86	0.42	10

culture 2 was observed to be different from those of the other cultures in that it was stimulated by low concentrations of oxytetracycline or of chloramphenicol (fig. 1). Growth-time studies employing the concentration of each antibiotic that gave maximal stimulation revealed that this stimulation was evident throughout the period of logarithmic growth but that on continued incubation a slight but definite increase in final growth was obtained in the tubes in which the antibiotic had been omitted (fig. 2).

The existence of this growth stimulation by either antibiotic in brain-heart broth, and its absence in the synthetic medium, prompted a search for the medium constituent(s) that led to this difference in reaction to the antibiotics. A partial resolution to the problem was obtained (fig. 3). As is evident, growth stimulation at intermediate concentrations of chloramphenicol was obtained when the medium was fortified with 0.5 per cent yeast extract (fig. 3, curve C) and the effect could then be increased if N-Z-Case amino acids were also present (curve D), although the latter was without effect if the yeast extract was omitted (curve B). Even with the medium corresponding to curve D the magnitude of the stimulation was less than that observed in brain-heart (curve E). Further investigation is required to elucidate whether this is a concentration effect or whether additional substances are required for the full effect. It is also desirable that the substances in yeast extract responsible for the observed effect be further identified.

*Effect of Chloramphenicol and of Oxytetracycline on the  $\beta$ -Galactosidase Activity of E. coli Mutants.* Preparatory to the induction of the adaptive enzyme an appropriate volume of the glucose salt medium was inoculated with  $\frac{1}{10}$  volume of an overnight culture of the organism and then incubated at 37 C., with forced aeration, until an optical density of 0.15 was reached. The cells were then centrifuged, washed with saline, and resuspended in a salt medium lacking a carbon

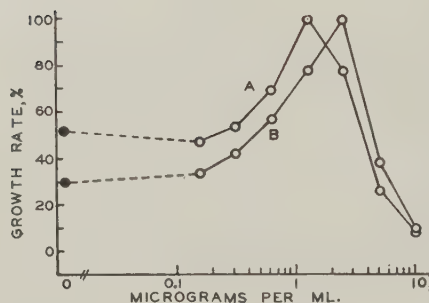
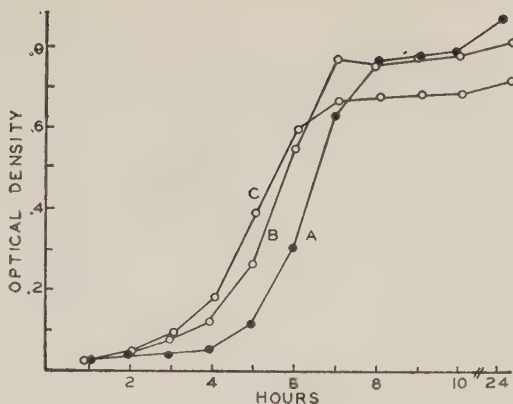


FIG. 1. Bacterial growth stimulation in brain-heart broth, as a function of the antibiotic concentration. Ordinates are the percentages of growth at each antibiotic concentration relative to that reached with the maximally stimulatory concentration. Curve A: growth in the presence of oxytetracycline; maximally stimulatory concentration = 1.25  $\mu\text{g.}$  Curve B: growth in the presence of chloramphenicol; maximally stimulatory concentration = 2.5  $\mu\text{g.}$  The full circles record the growth reached in each experiment in the absence of antibiotic; their position is naturally discontinuous with the log-drug abscissa scale.

FIG. 2. Stimulation of bacterial growth by antibiotics as a function of the time of incubation. Curve A: base medium (brain-heart broth) without antibiotic; Curve B: with addition of 1.25  $\mu\text{g.}$  oxytetracycline; Curve C: with addition of 2.5  $\mu\text{g.}$  chloramphenicol.



source. The suspension was adjusted so that a further 1:10 dilution would have an optical density of approximately 0.10.

For induction, 1 ml. of the suspended organisms was added to 9 ml. of the inducer solution (0.2 per cent melibiose<sup>2</sup> dissolved in minimal salt medium) to which the requisite amount of antibiotic was added. The suspension was then incubated at 37 C., with shaking, until growth reached the desired density.

The protein content of the entire culture was determined by the method of Lowry et al.,<sup>3</sup> utilizing Armour bovine serum albumin as a reference standard and human serum albumin as a working standard.  $\beta$ -Galactosidase activity was determined as described by Rickenberg and Lester,<sup>4</sup> except that reaction conditions of 10 minutes at 30 C. were utilized. The cell suspensions were prepared for enzyme assays by adding 10  $\mu\text{g.}$  of sodium desoxycholate and 0.02 ml. of toluene to the 1.0 ml. samples, which were then shaken at 37 C.

The specific activities for the seven cultures were first determined following induction in the absence of an antibiotic (table II). Since culture 1 appeared to have a significantly lower enzyme activity than the other resistant cultures it was selected for further study. In all instances parallel runs with the sensitive reference culture were made at the same time.

As an orientation, the specific activities of both cultures were determined at one incubation period following induction in the presence of melibiose and an antibiotic. By employing higher concentrations of the latter for the resistant strain roughly comparable degrees of growth inhibition could be secured for the two cultures (table III).

In a second set of experiments the induction times were varied so as to bring the optical densities of the cultures to the same level, in spite of varying degrees of growth inhibition by the antibiotic, i.e., all cultures were examined at the same

FIG. 3. Effect of medium on stimulation by chloramphenicol. Curve A: basal synthetic medium; Curve B: basal + 1 per cent amino acids; Curve C: basal + 0.5 per cent yeast extract; Curve D: basal + amino acids + yeast extract; Curve E: brain-heart broth. The ordinates are the percentages of the maximal growth reached with the optimal concentration of antibiotic. The latter were for Curves D and E, 2.5  $\mu\text{g.}$ ; Curve C, 1.2  $\mu\text{g.}$ ; Curve B, 0 drug. For Curve A the growth with 0.16  $\mu\text{g.}$  chloramphenicol was taken as the base, although this is probably not significantly different, experimentally, from that of O drug.

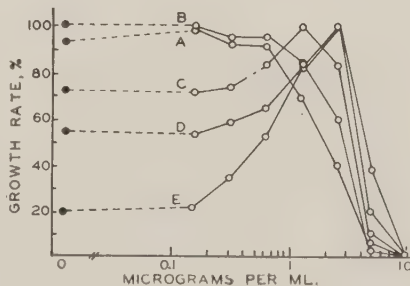


TABLE II

*β-Galactosidase Activities of Cultures Induced in Absence of Antibiotics*

Culture	Induction time, min.	Growth (optical density)	Protein $\mu\text{g.}/\text{ml.}$	ONPG* $\text{m}\mu\text{ M}$	Specific activity†	Relative specific activity
Parent, reference	175	0.25	380	8660	22.9	1.00
Resistant 1	265	0.19	280	1880	6.7	0.29
Resistant 2	180	0.26	394	7490	19.0	0.83
Resistant 3	205	0.24	418	8350	20.0	0.87
Resistant 4	185	0.25	400	7280	18.2	0.80
Resistant 5	200	0.25	390	6040	15.5	0.68
Resistant 6	220	0.25	316	3340	10.6	0.46

\* ONPG = ortho-nitrophenyl galactoside hydrolyzed per ml., per minute.

† Specific activity expressed as  $\text{m}\mu$  moles of *o*-nitrophenol released per minute, per  $\mu\text{g.}$  protein.

stage of the growth cycle. The effects on the specific activities are illustrated in table IV.

*Effect of Chloramphenicol and of Oxytetracycline on the D-Serine Deaminase Activities of E. coli Mutants.* Preparatory to the test the organisms were inoculated from stock slants into 10 ml. of a glycerine salt medium containing 0.5 per cent glucose<sup>6</sup> and incubated at 37 C., with shaking. For most cultures overnight incubation sufficed but slow-growing cultures required two days. On the day of the test the culture was diluted 1:10 in this medium and reincubated to an optical density of 0.11. To 9 ml. of the standardized culture were then added 1 ml. of the antibiotic solution and 0.1 ml. of a DL-serine solution (30 mg./ml.). The latter acted as an inducer of D-serine deaminase. The culture was then incubated at 37 C., with shaking, until the desired growth density was reached.

TABLE III

*Specific Activities of β-Galactosidases Formed in the Presence of Various Concentrations of Antibiotics and Assayed at the Same Induction Period*

Culture	Antibiotic concentration, $\mu\text{g.}/\text{ml.}$	Induction time, min.	Growth (optical density)	Protein $\mu\text{g.}/\text{ml.}$	ONPG* $\text{m}\mu\text{ M}$	Specific activity*	Relative specific activity
Controls Induced in Absence of Antibiotics							
Parent, reference	0	170	0.29	342	8350	24.4	1.00
Resistant 1	0	178	0.30	415	6680	16.1	1.00
Cultures Induced in Presence of Chloramphenicol							
Parent, reference	0.20	All	0.24	286	6530	22.8	0.93
	0.35	170	0.19	210	4750	22.6	0.93
	0.60		0.14	167	2100	12.6	0.52
Resistant 1	0.8	All	0.266	330	5860	17.8	1.10
	1.4	178	0.210	275	4620	16.8	1.04
	2.6		0.162	206	2570	12.5	0.78
Cultures Induced in Presence of Oxytetracycline							
Parent, reference	0.018	All	0.250	270	5910	21.8	0.89
		170					
	0.035		0.200	230	4010	17.4	0.71
Resistant 1	0.065		0.150	173	1930	11.2	0.46
	0.035	All	0.252	318	5180	16.3	1.01
		178					
	0.080		0.210	270	3450	12.8	0.80
	0.175		0.158	169	1330	7.9	0.49

\* See footnotes to table II.

TABLE IV

*Specific Activities of  $\beta$ -Galactosidases Formed in the Presence of Various Concentrations of Antibiotics and Assayed at the Same Growth Level*

Stock	Antibiotic concentration, $\mu\text{g./ml.}$	Induction time, min.	Growth (optical density)	Protein $\mu\text{g./ml.}$	ONPG* $\text{m}\mu\text{ M}$	Specific activity*	Relative specific activity
Controls Induced in Absence of Antibiotics							
Parent, reference	0	145	0.250	286	6420	22.4	1.00
Resistant 1	0	165	0.270	315	5780	18.4	1.00
Cultures Induced in Presence of Chloramphenicol							
Parent, reference	0.20	180	0.27	315	6960	22.1	0.99
	0.35	196	0.26	260	6530	25.1	1.12
	0.60	281	0.26	310	6460	20.8	0.93
Resistant 1	0.8	178	0.25	280	5690	20.3	1.10
	1.4	195	0.25	373	4900	13.1	0.71
	2.6	235	0.25	373	4880	13.1	0.71
Cultures Induced in Presence of Oxytetracycline							
Parent, reference	0.018	180	0.26	300	6810	22.7	1.01
	0.035	190	0.25	263	4880	18.6	0.83
	0.065	240	0.25	286	3770	13.2	0.59
Resistant 1	0.035	178	0.25	290	4760	16.4	0.89
	0.080	203	0.26	335	4110	12.3	0.67
	0.175	267	0.26	318	3150	9.9	0.54

\* See footnotes to table II.

The cells were washed, resuspended, and treated with sodium desoxycholate and toluene, with an aliquot for protein determination, as in the  $\beta$ -galactosidase assays. The D-serine deaminase activity was determined by the procedure of Pardee and Prestidge.<sup>6</sup> The specific activities are presented as the  $\text{m}\mu$  moles of  $\alpha$ -keto acid formed per minute per  $\mu\text{g.}$  protein.

As before, the specific activities were first obtained for each culture following induction in the absence of an antibiotic (table V). Culture 1 was again selected for further investigation. Table VI illustrates the effect of chloramphenicol and of oxytetracycline on the specific activities of the enzyme in experiments in which a fixed induction period was employed for each culture. In the final set of experiments the specific activities were determined at equal levels of growth for all cultures (table VII). In the last entry tabulated the D-serine deaminase activity of the reference culture is indetectable, in spite of the considerable cell growth.

TABLE V

*D-Serine Deaminase Activities of Cultures Induced in Absence of Antibiotics*

Culture	Induction time, min.	Growth (optical density)	Protein $\mu\text{g./ml.}$	D-Serine deaminated*	Specific activity†	Relative specific activity
Parent, reference	185	0.230	175	74.1	2.11	1.00
Resistant 1	205	0.197	150	40.7	1.36	0.64
Resistant 2	170	0.231	188	75.5	2.01	0.95
Resistant 3	210	0.205	172	70.3	2.03	0.96
Resistant 4	175	0.225	174	56.3	1.62	0.77
Resistant 5	210	0.203	179	86.9	2.44	1.16

\* As  $\text{m}\mu$  moles of pyruvate produced per minute per 0.2 ml. culture.

† Specific activity expressed as  $\text{m}\mu$  moles of  $\alpha$ -keto acid formed per minute per  $\mu\text{g.}$  of protein.

TABLE VI

*Specific Activity of D-Serine Deaminase Formed in the Presence of Antibiotics and Assayed at the Same Induction Period*

Culture	Antibiotic concentration, $\mu\text{g./ml.}$	Induction time, min.	Growth (optical density)	Protein $\mu\text{g./ml.}$	D-Serine deaminated*	Specific activity†	Relative specific activity
Controls Induced in Absence of Antibiotics							
Parent, reference	0	205	0.197	150	40.7	1.36	1.00
Resistant 1	0	185	0.222	145	35.1	1.21	1.00
Cultures Induced in Presence of Chloramphenicol							
Parent, reference	0.60	205	0.143	95	3.42	0.18	0.13
	1.2	205	0.148	84	0	0	0
Resistant 1	7.0	185	0.193	116	8.27	0.36	0.36
	14.0	185	0.170	102	3.14	0.15	0.13
Culture Induced in Presence of Oxytetracycline							
Parent, reference	0.08	205	0.160	102	5.98	0.29	0.22
	0.23	205	0.140	82	0	0	0

\* As  $\text{m}\mu$  of pyruvate produced per minute per 0.2 ml. culture.

† Specific activity expressed as  $\text{m}\mu$  moles of  $\alpha$ -keto acid formed per minute per  $\mu\text{g.}$  of protein.

## DISCUSSION

Although it is well established<sup>7</sup> that there is a considerable degree of cross resistance between chloramphenicol- and tetracycline-resistant gram-negative bacteria, the data of table I again illustrate that the crossing need not be quantitatively in proportion to the resistance to one of these antibiotics.

As in other instances of growth stimulation or of full dependence the special properties of culture 2 provide an opportunity of investigating further some of the

TABLE VII

*Specific Activities of D-Serine Deaminase Formed in the Presence of Antibiotics and Assayed at the Same Growth Level*

Culture	Antibiotic concentration, $\mu\text{g./ml.}$	Induction time, min.	Growth (optical density)	Protein $\mu\text{g./ml.}$	D-Serine deaminated*	Specific activity†	Relative specific activity
Controls Induced in the Absence of Antibiotics							
Parent, reference	0	205	0.200	152	44.2	1.45	1.00
Resistant 1	0	185	0.220	132	31.4	1.19	1.00
Cultures Induced in the Presence of Chloramphenicol							
Parent, reference	0.60	280	0.200	125	18.0	0.72	0.50
	1.2	435	0.210	130	16.0	0.62	0.42
	2.0	555	0.183	84	2.6	0.15	0.10
Resistant 1	7.0	220	0.220	146	19.1	0.65	0.55
	14	330	0.213	141	14.5	0.51	0.43
	25	395	0.220	125	12.8	0.51	0.43
Culture Induced in Presence of Oxytetracycline							
Parent, reference	0.08	245	0.199	137	13.1	0.48	0.34
	0.23	435	0.198	102	2.3	0.11	0.08
	0.50	555	0.150	64	0	0	0

\* As  $\text{m}\mu$  of pyruvate produced per minute per 0.2 ml. culture.

† Specific activity expressed as  $\text{m}\mu$  moles of  $\alpha$ -keto acid formed per minute per  $\mu\text{g.}$  of protein.

mechanisms involved in antibiotic action. Although the literature on the substances that reverse chloramphenicol and tetracycline actions is growing,<sup>8,9</sup> no common denominator is as yet evident. It is of interest, however, that both yeast extract and casein hydrolysate have been reported to have such actions; the relation of this finding to our observations on culture 1 is not clear but may become resolved as the chemical identities of the active materials are ascertained.

The inhibition of adaptive enzyme formation in representative systems by certain antibiotics, including chloramphenicol and tetracycline, is well known.<sup>8-10</sup> Our data extend the material available on at least partially characterized resistant bacteria, under conditions of potential growth. The data of table II indicate a considerable spread in the specific activities of the  $\beta$ -galactosidases induced in the various related stocks in the absence of antibiotics. The metabolic implications of this, in particular, the relations, if any, to resistance are not clear and require further investigation.

It would appear from the data of table III that the percentage depressions in enzymatic activities are roughly equal for the sensitive and resistant cultures when the comparisons are made at equal degrees of growth inhibition, but in each case the magnitudes are larger when oxytetracycline is used instead of chloramphenicol. The quantitation is altered somewhat when the comparisons are made at a constant, moderately high level of growth (table IV), although the greater inhibiting action of oxytetracycline is still evident.

The specific activities of D-serine deaminase, particularly those for cultures 4, 6, and 7, listed in table V are less widely spread from the mean than are those for  $\beta$ -galactosidase (table II). The interpretation of this provides an interesting problem the solution of which would require, however, extensive additional data on the influence of the growth cycle and differences in media. Since the inhibition of growth by each antibiotic was less in the medium used for the D-serine deaminase assays considerably higher antibiotic concentrations could be used.

The more specific inhibition of protein in contrast to nucleic acid synthesis by chloramphenicol and the tetracyclines has been noted by a number of investigators.<sup>8,9</sup> For our material this effect is presumably an important component in the diminishing ratios of protein to growth density at the higher drug concentrations, calculable from the data tabulated. In the most striking instances (as in the effect of oxytetracycline on the reference culture, table VII) the activity of the adaptive enzyme decreases far more rapidly than the total protein. Although the present data are suggestive that oxytetracycline, and to a lesser extent, chloramphenicol are more effective in suppressing D-serine deaminase activity than  $\beta$ -galactosidase activity a quantitative comparison is not justified here due to necessary differences in the experimental requirements of the two systems. This point, which is of obvious interest in connection with the mechanisms of adaptive enzyme formation and control, could best be investigated with other systems especially chosen for the purpose.

Although the point is somewhat obscured in the data reported in the tables, due to the fact that higher drug concentrations were employed in the experiments involving resistant organisms, it should be noted that culture 1, for example, did display its characteristic feature of antibiotic resistance since it continued to produce full amounts of enzyme at antibiotic concentrations that resulted in significant, if not complete, suppression of the enzymatic activity of the sensitive reference culture.

## BIBLIOGRAPHY

1. TREFFERS, H. P.: The linear representation of dosage-response curves in microbial-antibiotic assays, *J. Bact.* 72:108, 1956.
2. LESTER, G., AND BONNER, D.: The occurrence of  $\beta$ -galactosidase in *Escherichia coli*, *J. Bact.* 63:759, 1952.
3. LOWRY, O. H.; ROSEBROUGH, N. J.; FARR, A. L., AND RANDALL, R. J.: Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* 193:265, 1951.
4. RICKENBERG, H. V., AND LESTER, G.: The preferential synthesis of  $\beta$ -galactosidase in *Escherichia coli*, *J. Gen. Microbiol.* 13:279, 1955.
5. NOVICK, A., AND WEINER, M.: Enzyme induction as an all-or-none phenomenon, *Proc. Nat. Acad. Sc.* 43:555, 1957.
6. PARDEE, A. B., AND PRESTIDGE, L. S.: Induced formation of serine and threonine deaminases by *Escherichia coli*, *J. Bact.* 70:667, 1955.
7. WRIGHT, S. S., AND FINLAND, M.: Cross-resistance among 3 tetracyclines, *Proc. Soc. Exper. Biol. & Med.* 85:40, 1954.
8. EAGLE, H., AND SAZ, A. K.: Antibiotics, *Ann. Rev. Microbiol.* 9:173, 1955.
9. ETTLINGER, L.: Wirkungsmechanisms der Antibiotica, *In: Antibiotica et Chemotherapie*, Vol. 4. Basel, S. Karger, 1957, p. 60.
10. HAHN, F. E., AND WISSEMAN, C. L., JR.: Inhibition of adaptive enzyme formation by anti-microbial agents, *Proc. Soc. Exper. Biol. & Med.* 76:533, 1951.

# On the Design of Antibiotic Blood Level Experiments

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A basic problem facing the clinical investigator in evaluating new therapeutic agents is the wide variation observed among the subjects selected for testing. Even when clinical trials can be prearranged and controlled, as for example in the test of antibiotic blood levels among healthy volunteers, the between-patient and within-patient variances can sometimes mask real differences between the treatments under study.

For the past few years, many papers have been published reporting simulated clinical studies of the serum levels obtained on oral administration of various antibiotic formulations.

The experimental design favored by many workers is the "crossover" or Latin square wherein each subject receives each course of treatment once during the trial.

This design has the basic advantage of tending to eliminate patient-to-patient variations if there are no significant fluctuations from day to day in the experimental conditions and in the analytical technique and if the subjects have been properly randomized to their respective treatment groups.

Not all of the investigators reporting in this field have demonstrated a proper appreciation for experimental error. Furthermore, there is some question as to what factors, such as diet, have an effect on the experimental results.

## THE PROBLEM

It is the purpose of this work to elucidate the importance of some of the factors involved in the design of antibiotic blood level experiments. In this paper we present the design, the experimental procedure, the analysis of the data, and the interpretation of the results of a human blood level experiment with tetracycline hydrochloride, with and without adjuvants.

## DESIGN OF THE EXPERIMENT

*Antibiotics.* Samples of commercial lots of three antibiotic-adjuvant combinations were used in this investigation. They were tetracycline hydrochloride, tetracycline hydrochloride plus glucosamine hydrochloride, and tetracycline hydrochloride plus citric acid.

Although it has not been demonstrated that small variations in the antibiotic content of commercial capsules yield corresponding variations in human blood levels, care was taken to eliminate this as a factor in the experiment.

Grab samples were taken at random from each of the three lots. Each capsule in the sample was weighed individually. Those capsules whose weights were  $\pm 2$  per cent variation from the expected gross weight of the capsule were culled out. Five capsules were then selected at random from each of the remaining batches of capsules and submitted for bioassay. These were assayed using *Klebsiella pneumoniae* (PCR-602) with the turbidimetric method described by Kersey.<sup>3</sup> As can be seen from table I, there was no significant difference in antibiotic activity between the commercial lots selected.

*Diet.* As a second factor in the experiment, three conditions of diet were im-

TABLE I  
*Bioassay of Capsule Samples (mg./capsule)*

	Antibiotic		
	Tetracycline hydrochloride	Tetracycline with glucosamine	Tetracycline hydrochloride with citric acid
Code number	81-156-71 EPD	81-136-71 EPD	81-135-73 EPD
Source	Commercial lot	Commercial lot	Commercial lot
Assays	254 241 237 258 243	237 271 242 250 244	260 245 247 233 252
Average	246.6	248.8	247.4
Total capsules	5	5	5
Standard deviation	±8.96	±13.26	±9.91

posed. The first one was a partial fast wherein subjects did not consume any solid food or liquids other than water 12 hours prior to receiving the antibiotic. The second condition was similar to the first except that the subject received 9 Gm. of emulsified linoleic acid simultaneously with the antibiotic dose. The third condition permitted the subject to consume breakfast ad libitum 0.5 hour prior to dosage time.

*Selection of Subjects.* Sixty healthy men, ranging in weight from 135.5 to 260.0 lb., were assigned numbers in descending order of weight. The first 20 subjects were designated heavyweights, the next 20 middleweights, and the last 20 lightweights. Subjects 19, 20, 21, 40, 41, and 42 were dropped from the experiment to create boundaries between the weight groups. No subject was on antibiotic therapy for at least four days prior to the experiment. Blood samples taken prior to dosage were assayed to check for antibiotic carryover. All subjects were negative.

*Assignment of Treatments.* Within each weight group, each subject was assigned to a treatment pair in the order in which his number appeared in a table of random numbers. Each treatment pair was then assigned to a specific antibiotic and diet treatment on the first day of the experiment. On the fourth and seventh days the treatment pairs were crossed over to a different antibiotic-diet treatment. This procedure permitted all subjects to receive all antibiotics and all diets some time during the course of the investigation.

The design of the experiment is shown in table II. The experiment was arrayed in a factorial design involving four factors (antibiotics, diets, weight group, and days) at three levels. Within weight groups the treatment pairs formed subject teams, which were rotated from treatment to treatment in a Latin square design.

#### PROCEDURE

The subjects received a single 250 mg. capsule on each treatment day. The subjects were treated in their body weight order. Three hours after treatment, serum samples were drawn from the subjects in the same order. Three days' rest was permitted between treatments to minimize the possibility of antibiotic carryover.

The blood samples were centrifuged and the supernatant liquors were assayed using *Bacillus cereus* var. *mycoides* (ATCC 9634) by the plate method described by Grove and Randall.<sup>2</sup> Where any delay was expected between sampling and assay, the sera were stored in a frozen state.

TABLE II  
Design of Experiment and Assignment of Treatments

Day	Diet†	Group I*						Group II						Group III					
		Tetracycline			Tetracycline plus glucosamine			Tetracycline plus citric acid			Tetracycline plus glucosamine			Tetracycline plus citric acid			Tetracycline plus glucosamine		
		Pair	Subj.	Pair	Subj.	Pair	Subj.	Pair	Subj.	Pair	Subj.	Pair	Subj.	Pair	Subj.	Pair	Subj.	Pair	Subj.
First (1-15-58)	F	Team A			Team B			Team C			Team P			Team Q			Team R		
		a	3, 16	b	12, 18	c	11, 14	c	11, 14	c	33, 36	b	24, 26	c	27, 32	a	52, 57	b	45, 51
	FF	d	5, 7	e	9, 17	f	1, 8	f	1, 8	f	37, 38	e	31, 35	f	23, 30	d	43, 59	e	44, 49
Fourth (1-19-58)	N	Team A			Team B			Team C			Team P			Team Q			Team R		
		a	10, 15	h	4, 13	j	2, 6	j	2, 6	j	22, 34	h	25, 39	j	28, 29	g	46, 58	h	48, 56
Seventh (1-22-58)	F	Team A			Team B			Team C			Team P			Team Q			Team R		
		f	1, 8	d	5, 7	e	9, 17	e	9, 17	e	23, 30	d	37, 38	e	31, 35	f	47, 54	d	43, 59
	FF	j	2, 6	g	10, 15	h	4, 13	h	4, 13	h	28, 29	g	22, 34	h	25, 39	j	50, 53	g	46, 58
Tenth (1-25-58)	N	Team A			Team B			Team C			Team P			Team Q			Team R		
		a	11, 14	a	3, 16	b	12, 18	b	12, 18	b	27, 32	a	33, 36	b	24, 26	c	55, 60	a	52, 57
Thirteenth (1-28-58)	F	Team A			Team B			Team C			Team P			Team Q			Team R		
		h	4, 13	j	2, 6	g	10, 15	g	10, 15	g	25, 39	j	28, 29	g	22, 34	h	48, 56	j	50, 53
	FF	b	12, 18	c	11, 14	a	3, 16	a	3, 16	a	24, 26	b	27, 32	a	33, 36	b	45, 51	c	55, 60
Sixteenth (1-31-58)	N	Team A			Team B			Team C			Team P			Team Q			Team R		
		e	9, 17	f	1, 8	d	5, 7	d	5, 7	d	31, 35	e	37, 38	e	31, 35	f	47, 54	e	44, 49

\* Group I—heavyweights, ranging from 181.0 to 260.0 lb.; Group II—middleweights, ranging from 161.5 to 178.0 lb.; Group III—lightweights, ranging from 135.5 to 159.0 lb.

† Diet code: F—fasting for 12 hours before dosage; FF—same as F but consumed 9 Gm. linoleic acid at dosage time; N—consumed full meal ad libitum 0.5 hour before dosage time.

TABLE III  
*Results of Antibiotic Blood Level Experiment*

Subj. no.	Group no.	Pair no.	Age, yr.	Weight, lb.	Height, inches	First Day		Fourth Day		Seventh Day		Blood level, 3 hr.		
						Drug*	Diet†	Blood level, 3 hr.	Drug	Diet	Blood level, 3 hr.		Drug	Diet
1	I	f	43	260.00	67.250	TC	FF	1.100	T	F	1.645	TG	N	0.314
2	I	j	33	235.00	73.000	TC	N	0.710	T	FF	1.685	TG	F	2.015
3	I	a	40	229.00	74.250	T	F	0.890	TG	N	0.368	TC	FF	1.695
4	I	h	21	226.00	70.000	TG	N	0.735	TC	FF	2.340	T	F	1.455
5	I	d	27	222.50	67.500	T	FF	1.815	TG	F	2.145	TC	N	0.985
6	I	j	35	219.00	72.000	TC	N	0.750	T	FF	2.055	TG	F	1.805
7	I	d	22	214.00	73.000	T	FF	1.200	TG	F	0.760	TC	N	0.580
8	I	f	33	206.00	69.500	TC	FF	1.060	T	F	1.445	TG	N	0.635
9	I	e	23	202.00	72.125	TG	FF	2.270	TC	F	2.290	T	N	0.760
10	I	g	53	193.00	69.000	T	N	0.336	TG	FF	1.500	TC	F	2.540
11	I	c	24	190.50	69.250	TC	F	1.245	T	N	0.306	TG	FF	1.655
12	I	b	26	185.00	70.500	TG	F	2.000	TC	N	0.505	T	FF	2.255
13	I	h	22	186.00	73.000	TG	N	0.535	TC	FF	1.800	T	F	1.890
14	I	c	43	184.50	66.000	TC	F	1.660	T	N	1.405	TG	FF	2.310
15	I	g	38	182.00	69.500	T	N	0.565	TG	FF	1.385	TC	F	2.015
16	I	a	41	181.00	69.500	T	F	1.895	TG	N	1.095	TC	FF	1.970
17	I	e	21	181.00	73.000	TG	FF	1.635	TC	F	1.755	T	N	0.950
18	I	b	33	181.00	70.125	TG	F	1.690	TC	N	0.685	T	FF	2.310
22	II	g	39	178.00	67.500	T	N	1.155	TG	FF	2.100	TC	F	1.890
23	II	f	22	178.00	71.000	TC	FF	0.830	T	F	1.800	TG	N	1.185
24	II	b	45	177.50	66.000	TG	F	2.585	TC	N	0.760	T	FF	2.935
25	II	h	43	177.25	70.750	TG	N	1.600	TC	FF	1.800	T	F	2.865
26	II	b	48	173.00	66.750	TG	F	3.340	TC	N	1.570	T	FF	2.205
27	II	c	27	173.00	68.000	TC	F	1.510	T	N	0.795	TG	FF	2.205
28	II	j	32	168.00	71.000	TC	N	0.685	T	FF	1.010	TG	F	2.160
29	II	j	30	167.00	69.000	TC	N	0.410	T	FF	1.755	TG	F	2.310
30	II	f	28	166.50	73.500	TC	FF	1.690	T	F	1.385	TG	N	0.730
31	II	e	23	166.00	70.250	TG	FF	1.750	TC	F	0.970	T	N	1.455
32	II	c	20	165.50	71.750	TC	F	1.460	T	N	0.204	TG	FF	2.015
33	II	a	26	165.25	67.250	T	F	2.085	TG	N	2.100	TC	FF	2.310
34	II	g	25	165.00	69.000	T	N	1.100	TG	FF	2.890	TC	F	2.365
35	II	e	23	165.00	68.750	TG	FF	2.045	TC	F	3.005	T	N	1.340
36	II	a	24	164.50	62.250	T	F	2.270	TG	N	1.140	TC	FF	1.735
37	II	d	22	163.50	69.250	T	FF	1.685	TG	F	2.005	TC	N	1.615
38	II	d	22	162.50	70.500	T	FF	2.340	TG	F	3.005	TC	N	0.780
39	II	h	19	161.50	70.250	TG	N	0.905	TC	FF	2.240	T	F	1.970
43	III	d	35	159.00	68.000	T	FF	1.290	TG	F	1.715	TC	N	0.438
44	III	e	22	158.00	68.500	TG	FF	1.600	TC	F	1.920	T	N	1.430
45	III	b	21	156.50	68.500	TG	F	2.365	TC	N	0.481	T	FF	2.060
46	III	g	28	155.00	69.750	T	N	1.720	TG	FF	2.290	TC	F	0.125
47	III	f	35	155.00	68.250	TC	FF	1.660	T	F	2.710	TG	N	1.060
48	III	h	32	152.00	70.000	TG	N	1.305	TC	FF	2.140	T	F	0.985
49	III	e	34	152.00	65.750	TG	FF	1.930	TC	F	2.390	T	N	1.005
50	III	j	27	151.00	71.000	TC	N	0.800	T	FF	1.500	TG	F	1.255
51	III	b	32	150.50	71.750	TG	F	2.760	TC	N	1.470	T	FF	2.365
52	III	a	26	148.00	68.750	T	F	1.245	TG	N	0.950	TC	FF	2.540
53	III	j	24	146.00	64.250	TC	N	1.060	T	FF	2.655	TG	F	3.790
54	III	f	44	145.75	67.500	TC	FF	1.385	T	F	1.845	TG	N	0.970
55	III	c	38	145.00	70.750	TC	F	2.180	T	N	0.620	TG	FF	2.660
56	III	h	46	145.00	70.500	TG	N	1.895	TC	FF	2.140	T	F	1.310
57	III	a	39	142.00	65.250	T	F	1.245	TG	N	0.331	TC	FF	1.285
58	III	g	34	138.00	66.500	T	N	1.040	TG	FF	2.195	TC	F	2.795
59	III	d	26	138.00	69.250	T	FF	1.660	TG	F	2.655	TC	N	0.970
60	III	c	22	135.50	65.750	TC	F	1.750	T	N	0.457	TG	FF	2.865

\* T—tetracycline; TG—tetracycline plus glucosamine; TC—tetracycline plus citric acid.

† F—fasting for 12 hours before dosage; FF—same as F but consumed 9 Gm. linoleic acid at dosage time; N—consumed full meal ad libitum 0.5 hour before dosage time.

TABLE IV  
General Analysis of Variance

Sources of variance	Degrees of freedom	Sums of squares	Mean squares	F ratio against R	Component variances of the main effects and interactions																			
					d	e	f	g	de	df	dg	ef	eg	fg	def	deg	dfg	efg	defg	r				
Main effects																								
Antibiotics (D)	2	2.3538	1.1769	4.33°	x					o	o	o				o	x	o		o	x			
Days (E)	2	1.1602	0.5801	2.13		o				o			o	o			o	x		o	o	x		
Diets (F)	2	37.3462	18.6731	68.67†			x				o				o				o	o	o	x		
Weight groups (G)	2	3.5716	1.7858	6.57†				x				o			o	o		x	o	o	o	x		
First order interactions																								
D x E	4	2.4483	0.6121	2.25						o							o	x			o	x		
D x F	4	1.3040	0.3260	1.20							o							o		o		o	x	
D x G	4	1.6599	0.4150	1.53								o						x	o			o	x	
E x F	4	1.8420	0.4605	1.69									o							o		o	x	
E x G	4	0.4791	0.1198	0.44										o				x		o		o	x	
F x G	4	0.3615	0.0904	0.33											o					o	o	o	x	
Second order interactions																								
D x E x F	8	1.1074	0.1384	0.51													o					o	x	
D x E x G	8	4.5456	0.5682	2.09°														x				o	x	
D x F x G	8	0.7649	0.0956	0.35																o			o	x
E x F x G	8	2.2068	0.2759	1.01																	o		o	x
Third order interactions																								
D x E x F x G	16	1.8915	0.1182	0.43																			o	x
Residual																								
R	81	22.0250	0.2719	—																				x
Total																								
T	161	85.0678	—	—																				

o—indicates that component does not exist on the basis of the F ratio test against the residual; x—indicates that component does exist on the basis of the same test.<sup>1</sup>

Pooling all main effects and interactions having the same x patterns, two pooled estimates of error are obtained:  $R_1 = (E, DE, DG, EG, \text{ and } DEG \text{ pooled}) = 0.4679$ .  $R_2 = (DF, EF, FG, DEF, DFG, EFG, DEFG, \text{ and } R \text{ pooled}) = 0.2369$ .

° Significant.

† Highly significant.

## OBSERVATIONS

The results obtained are summarized in table III in the order in which the subjects were treated. The blood level values shown are in  $\mu\text{g.}/\text{ml.}$  of serum.

These data were reduced by the analysis of variance. The results of this procedure are shown in table IV.

## ANALYSIS OF THE DATA

*Experimental Error.* The first estimate of experimental error derived was the residual error, which reflects the individual variation between the 27 pairs of subjects treated alike over all the conditions of the experiment. It should be noted that this value, 0.2719, yields a coefficient of variation of 32 per cent, which indicates that this type of data has an inherently high variability.

In testing the main effects and interactions against the residual error, variances due to antibiotic formulations, dietary conditions, weight groups, and the antibiotic-day-weight group interaction were found to be significant. Those main effects or interactions having the same significant components of variances were pooled to yield two improved estimates of experimental error,<sup>1</sup>  $R_1$  and  $R_2$ . Since estimate  $R_1$  refers to drugs and weight groups, it is the proper estimate to use in orthogonal comparisons<sup>4</sup> between treatments within these factors. The orthogonal comparison

TABLE V  
*Orthogonal Comparisons between Antibiotics*

	Tetracycline hydrochloride	Tetracycline hydrochloride with glucosamine	Tetracycline hydrochloride with citric acid
Code designation	T	TG	TC
Number of subjects	54	54	54
Sum of all serum assays	82.92	95.57	80.84
Arithmetic mean	1.535	1.770	1.497
	Student's t values		
Estimate of error used	0.4679 (see table IV.)		
Degrees of freedom	18		
	Comparison	Computed t	Conclusion
	TG vs. T	1.77	Significant
	TG vs. TC	2.07	Significant
	TC vs. T	0.29	Not significant

between drugs is shown in table V. Here we see that the average blood level achieved with tetracycline hydrochloride plus glucosamine over all the conditions of the experiment was significantly higher than the blood levels achieved with either tetracycline hydrochloride or tetracycline hydrochloride plus citric acid.

A similar comparison between weight groups reveals that the average blood level achieved by all antibiotics within the heavyweight group was significantly lower than the blood levels achieved by all antibiotics within the other two weight groups. The highly significant effect of the dietary conditions is obvious from an examination of the original data and from the comparisons in table VI.

Since the antibiotic-day-weight group interaction was significant and since the diet factor did not participate in any significant interactions, the data were pooled by subject teams and reduced by analysis of variance within weight groups. The results of these computations are summarized in table VII. Within all groups, the day factor was insignificant, indicating that variations in technique and the procedures from day to day were under control, i.e., within the limits of experimental error. There was a significant difference between antibiotics within both the middleweight and lightweight groups, but not within the heavyweight group. There was a significant difference between subject teams within the middleweight group, but not within the other two weight groups.

TABLE VI  
*Comparison of Average 3 Hour Blood Levels under Different Dietary Conditions*

Tetracycline formulation	Dietary conditions		
	After breakfast, average $\mu\text{g.}/\text{ml.}$	After 9 Gm. emulsified linoleic acid, average $\mu\text{g.}/\text{ml.}$	Fasting, average $\mu\text{g.}/\text{ml.}$
Tetracycline hydrochloride (no adjuvant)	0.93	1.96	1.73
	(control)		
Tetracycline hydrochloride with citric acid	0.85	1.76	1.88
Tetracycline hydrochloride with glucosamine	0.99	2.07	2.24

TABLE VII  
Analysis of Variance within Weight Groups

Group I (Heavyweights)											
Antibiotic							Degrees				
T		TG		TC			Sources of variance	of freedom	Sums of squares	Mean squares	F ratio
Day	Team	Σ Assays	Team	Σ Assays	Team	Σ Assays					
First Fourth Seventh	A C B	6.72 8.57 9.63	B A C	8.88 7.27 8.74	C B A	6.53 9.39 9.79	Antibiotics	2	0.1442	0.0721	—
							Days	2	6.0618	3.0309	2.45
							Teams	2	3.7180	1.8590	1.50
							Residual	2	2.4715	1.2358	—
							Total	8	12.3955	—	—

Group II (Middleweights)											
Antibiotic							Degrees				
T		TG		TC			Sources of variance	of freedom	Sums of squares	Mean squares	F ratio
Day	Team	Σ Assays	Team	Σ Assays	Team	Σ Assays					
First Fourth Seventh	P R Q	11.12 6.96 12.79	Q P R	12.24 13.25 10.62	R Q P	6.55 10.35 10.71	Antibiotics	2	12.2595	6.1298	9.06 <sup>o</sup>
							Days	2	3.4245	1.7123	2.53
							Teams	2	27.3950	13.6975	20.24 <sup>o</sup>
							Residual	2	1.3538	0.6769	—
							Total	8	44.4328	—	—

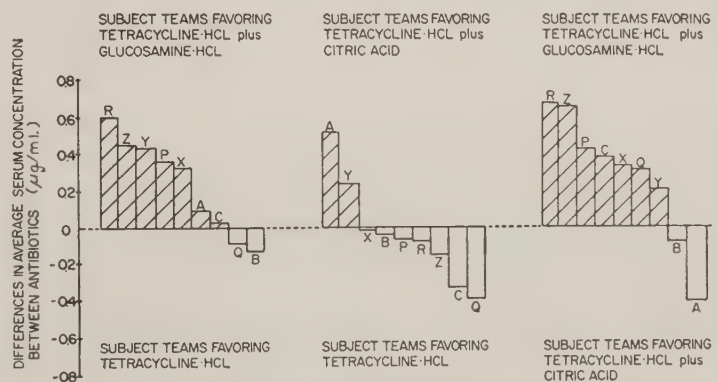
Group III (Lightweights)											
Antibiotic							Degrees				
T		TG		TC			Sources of variance	of freedom	Sums of squares	Mean squares	F ratio
Day	Team	Σ Assays	Team	Σ Assays	Team	Σ Assays					
First Fourth Seventh	X Z Y	8.21 9.80 9.17	Y X Z	11.84 10.15 12.61	Z Y X	8.84 10.54 8.17	Antibiotics	2	11.6551	5.8276	6.83 <sup>†</sup>
							Days	2	0.4417	0.2209	—
							Teams	2	5.2854	2.6427	3.10
							Residual	2	1.7072	0.8536	—
							Total	8	19.0894	—	—

\* Highly significant.  
† Significant.

### INTERPRETATION

The experiment revealed that the weight of the subjects can be a complicating factor in such investigations. If body weight is a rational criterion for randomizing subjects to treatment groups, the average blood levels for teams within weight groups should not show significant differences. The existence of a significant interaction traceable in part to the variation of teams within groups indicates a lack of homogeneity. This disparity is emphasized further by the comparison illustrated in figure 1. Here a particular team's average blood level with one antibiotic-adjuvant com-

FIG. 1. A comparison of antibiotic blood level averages by subject teams is shown.



bination is compared to the same team's average blood level with another antibiotic-adjuvant combination.

Finally, attempts to correlate individual subjects' blood levels with their body weights led to the conclusion that there is no relationship other than that heavy-weight people as a group tend to show lower blood levels.

This situation, however, can lead to serious bias in any experiment where subjects are randomized into treatment blocks on the basis of body weight. As the results of this experiment show, it is highly probable that such a randomization process could lead to teams composed of high-absorbing or low-absorbing individuals. As figure 1 demonstrates, even though we had randomized our subjects into weight groups and teams within weight groups, there was a considerable variation in response between groups of subjects. If our day-to-day variation in assay and experimental technique had been significant in this experiment, this variation between subject teams within weight groups would have caused a serious bias against one of the particular treatments. However, as we have seen from the analysis of variance, the between-day variance was insignificant. Hence, this experiment provides an unbiased comparison between antibiotics and diet treatments.

It would appear that if the subjects were classified by their ability to absorb an antibiotic prior to undertaking an investigation, the investigator would possess a more rational basis for randomization. Subjects could be treated with a control and ranked. They would then be randomized to treatment blocks. The process could then be tested for bias prior to beginning the experiment by comparing the rank totals of each team.<sup>5</sup> With the assurance that between-team variances are minimized, the experimenter has available designs that are more efficient and flexible than the classical crossover.

#### SUMMARY

The investigation revealed that randomization of subjects on the basis of body weight will probably group the subjects into high- and low-absorber teams. The results of a blood level experiment so organized might tend to be biased when variations in technique are uncontrolled. Pretesting subjects for their absorption ability and assigning them to treatments on this basis rather than by body weight are suggested.

The experiment described in this report produced an unbiased comparison between treatments because the variance attributable to day-to-day fluctuations in technique was insignificant.

The blood levels obtained with a single oral dose of tetracycline hydrochloride with glucosamine were significantly higher than those obtained with a single oral dose of tetracycline hydrochloride or tetracycline hydrochloride with citric acid.

#### BIBLIOGRAPHY

1. BROWNLEE, K. A.: *Industrial Experimentation*, ed. 4 (American), New York, Chemical Publishing Company, 1953, p. 92 ff.
2. GROVE, D. C., AND RANDALL, W. A.: *Assay Methods of Antibiotics: A Laboratory Manual*, New York, Medical Encyclopedia, Inc., 1955.
3. KERSEY, R. C.: A turbidimetric assay for Terramycin, *J. Am. Pharm. A.* 39:252, 1950.
4. OSTLE, B.: *Statistics in Research*, ed. 1, Ames, Iowa, The Iowa State College Press, 1954, p. 267 ff.
5. WILCOXIN, F.: Individual comparisons by ranking methods, *Biometric Bull.* 1:80, 1945.

# Numerical Designation of Staphylococcal Antibigrams

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Several workers<sup>1-3</sup> have reported the use of antibigrams in identifying strains of staphylococci. Such use is increasing, for antibigrams are clinically essential and are epidemiologically useful where phage typing is not available. Oeding and Sompolinsky,<sup>4</sup> however, in a study of the relationship between phage patterns, serology, and antibigrams, reported "generally good correlation between the results obtained by all the different methods." The accuracy of the antibigrams in the identification of strains may be increased by extending the list of antimicrobial agents used (barring similar modes of action and cross resistance), but the number of possible antibigrams increases exponentially ( $C = 2^n$ ). Thus, with only five antibiotics used there are 32 possible antibigrams. The naming of all the antibiotics used, however, plus stating the reaction of the bacteria to each antibiotic, is cumbersome whether written or spoken. A scheme is herewith proposed for a numerical designation of antibigrams.

## METHOD

A mnemonic, SPECT, is used for the five antibiotics (streptomycin, penicillin, erythromycin, chloramphenicol, and tetracycline) most commonly used for antibigrams. By assigning each antibiotic a number (1, 2, 3, 4, 5) according to its position in the mnemonic SPECT, resistance can be denoted by use of the number, susceptibility by its omission. Staphylococci susceptible to all five antibiotics are designated as antibigram zero (Abg 0). Those resistant to penicillin only are Abg 2. A common "epidemic" strain from hospitals is Abg 125. This system may be expanded for additional antibiotics by using suffixes. Thus, a *Staphylococcus* resistant to penicillin and tetracycline but susceptible to streptomycin, erythromycin, chloramphenicol, kanamycin, and vancomycin is Abg 25KV. If identical, except resistant to the last two, it is Abg 25K6V7.

## DISCUSSION

Several schemes have been used by various authors. Some have listed the different antibigrams observed and assigned them numbers.<sup>5,6</sup> Others have used abbreviations, usually the initial letter in the antibiotic name.<sup>1</sup> Both of these are difficult to use in conversation.

Problems arising out of the use of various forms of the antibiotics employed in the antibigram can be easily resolved. Most of the different forms of a given antibiotic (e.g., penicillin O, K, or G; tetracycline hydrochloride or phosphate) give similar if not identical results in in vitro susceptibility testing. Should an author wish to denote, for example, the use of oxytetracycline instead of tetracycline in the antibigram, the brief explanation (T = oxytetracycline) would suffice. It is felt that five antibiotics represent the optimum number for a basic designation in conversation, and the use of additional antibiotics should be denoted by letters. The five antibiotics chosen have been widely used and show adequate variation from susceptibility to resistance. Some antibiotics, e.g., neomycin, almost invariably inhibit

staphylococci<sup>7,8</sup> so that they are not suitable "markers." Others are so similar in action or have sufficient cross resistance that they show little promise as useful antibiogram components when identifying strains. However, as antibiotics do become recognized and accepted as additional "markers," they could be approved by a suitable commission in the same manner that phage type designations are handled.

This system offers the following advantages: (1) The more common types, susceptible to several antibiotics, have numbers of few digits, and only the rarer, multiple resistant organisms have large numbers. (2) Specific antibiotics may be quickly located in a long array of possible combinations because of the fixed sequence of the antibiotics in the mnemonic. (3) A reference to any of the 32 antibiograms can be readily deciphered without using a table or code chart. Learning the mnemonic, not memorizing the antibiograms by number, is the only requirement. (4) Expansion of this system is possible without changing the basic designations.

#### SUMMARY

A numerical designation of staphylococcal antibiograms is proposed in which antibiotics are assigned a number in the mnemonic SPECT. Resistance is denoted by use of the number, susceptibility by its omission. The reasons for choosing this system and its advantages are discussed.

#### BIBLIOGRAPHY

1. DUKES, C. D., AND DUKES, A. M.: The utilization of multiple resistance to antibiotics as an aid to determining cross-infection with *Staphylococcus aureus*. In: Antibiotics Annual 1953-1954, New York, Medical Encyclopedia, Inc. 1954; pp. 314-317.
2. LEPPER, M. H.; JACKSON, G. G., AND DOWLING, H. F.: Usefulness of minimal inhibitory concentrations of antibiotics to identify strains of *Micrococcus pyogenes* var. *aureus*. In: Antibiotics Annual 1954-1955, New York, Medical Encyclopedia, Inc. 1955; pp. 1037-1045.
3. SOMPOLINSKY, D.; HERMANN, Z.; OEDING, P., AND RIPON, J. E.: A series of postoperative infections, J. Infect. Dis. 100:1, 1957.
4. OEDING, P., AND SOMPOLINSKY, D.: On typing of *Staphylococcus aureus*: The relationship between typing according to phage patterns, serology, and antibiograms, J. Infect. Dis. 102:23, 1958.
5. BONDI, A.; PFAFF, F.; FREE, E., AND SWERLICK, R.: Public health aspects of the development of antibiotic-resistant staphylococci, Am. J. Pub. Health 44:879, 1954.
6. VACCARO, H.; PAREDES, L., AND VALENZUELA, E.: In vitro sensitivity of 300 strains of *Micrococcus pyogenes* isolated from carriers to seven antibiotics. In: Antibiotics Annual 1955-1956, New York, Medical Encyclopedia, Inc. 1956; pp. 623-633.
7. GREER, J. E., AND MENARD, R. R.: Neomycin susceptibility for differentiating coagulase-positive from coagulase-negative staphylococci. In: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc. 1958; pp. 149-153.
8. GREER, J. E.; MENARD, R. R., AND LESNEY, P. F.: Antibiotic concentrations in discs used for susceptibility testing of staphylococci. In: Antibiotics Annual 1958-1959, New York, Medical Encyclopedia, Inc. 1959; p. 818.

# Visual Detection of Antibiotics in Milk by Means of a Dye

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Antibiotics used for mastitis therapy are secreted in milk. Recent surveys carried out by several university and government groups<sup>1-4</sup> have established that 1 to 11 per cent of the nation's milk supplies contain antibiotics. The presence of antibiotics in milk usually renders the milk unsuitable for the manufacture of cultured dairy products. Also, the health officials have indicated that when ingested through milk, penicillin might produce ill-effects in "exquisitely sensitive" human beings. In order to reduce the economic losses to the manufacturer and to avoid the development of possibly harmful effects to the public, the Food and Drug Laws require that the milk from treated animals be withheld from the market for 72 hours after the last treatment. The 72 hour withholding period is sometimes more than adequate and sometimes less than adequate to keep antibiotic-milk out of the market.

There exists a need for rapid methods of detecting the presence of antibiotics in milk. Such methods could be used advantageously by the manufacturer and the health official. The methods would also be welcomed by the milk producer in order that he need not discard milk from treated cows for any longer than is necessary.

There are available several sensitive and accurate methods for the determination of antibiotics in milk, but almost all of them require several hours for completion.<sup>4-6</sup> One of the means proposed for a more rapid detection of antibiotics in milk has been the introduction of tracers like dyes, radioactive chemicals, or fluorescent compounds in the antibiotic preparations, which could be traced easily in milk and could give an indirect measure of the antibiotics in milk. Dalgaard-Mikkelsen and Rasmussen<sup>7</sup> reported that when the green S dye (monosodium salt of 4, 4-dimethylaminophenyl)-2-hydroxy-6, 8-disulphonaphthyl-carbinol-anhydride), mixed with a penicillin preparation, was infused into goats' and cows' udders, the milk secreted possessed a green color that corresponded well with the penicillin concentration in the milk. Hargrove et al<sup>8</sup> have shown that the infusion of fat soluble fluorescein and uranine, along with penicillin, resulted in the secretion of the "Marker" in milk, which gave an indirect measure of the antibiotics in milk. The present study was conducted to screen several dyes for use as markers and to standardize techniques for a satisfactory method for the visual detection of antibiotics in milk.

## EXPERIMENTAL

Preliminary trials were conducted with a few edible colors, vegetable extracts, and dyes to determine if they could lend themselves to use for visual detection of antibiotics in milk. Except for a green-turquoise dye, no other compound offered promise for use, since they had to be present in considerably high concentrations before they could be detected. The green-turquoise dye and commercially available penicillin G procaine preparations in oil and in aqueous suspensions were used throughout this study.

The dye was weighed accurately as aseptically as possible and dissolved in sterile

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pyrogen-free water. Penicillin in aqueous suspension also was diluted with the pyrogen-free water, whenever necessary. Known quantities of the antibiotic and of the dye were mixed in a sterile hypodermic syringe and infused into the udder by means of a sterile milk tube. Young, healthy lactating animals from the University herd were used in this work. The antibiotic treatment was always given immediately following the afternoon milking. No special care was provided for the experimental animals and they were allowed to mix with the rest of the animals as usual.

Following the treatment, milk samples were collected from each quarter at each regular milking time (4:00 a.m. and 4:00 p.m.) and assayed for penicillin and color intensity. The antibiotic concentrations were determined by the disc assay method employing whey agar as the assay medium and *Bacillus subtilis* spore suspension as the test organism. The color intensity was measured visually in the whole milk, and photometrically in a serum prepared from the milk. For visual color measurement, one part of milk was diluted with a normal mixed herd milk supply until the green-turquoise color was no longer perceptible to the naked eye. For ease of discussion, the number of parts of normal milk required to dissipate the color in one part of the colored milk is termed the "visibility index." For spectrophotometric determinations of the color intensity, several methods were used, and the following procedure seemed to give reasonably good results. It involved preparation of a clear serum from the colored milk and comparing it in a spectrophotometer with a serum prepared from a normal milk. In a 50 ml. flask, 10 ml. of the colored milk were mixed with 40 ml. of 15 per cent trichloroacetic acid. The contents were mixed well and after 10 minutes filtered through a dry filter paper. The color in the filtrate was measured at 640  $m\mu$  in a Coleman spectrophotometer model 14, the instrument having been adjusted to 100 per cent transmission with a blank solution prepared from normal milk. The wavelength of 640  $m\mu$  was selected because a standard curve prepared with a range of color concentrations apt to be encountered in the study showed an agreement with Beer's law at 625 to 660  $m\mu$ .

Several trials without the antibiotic were conducted to establish the relationship between the quantity of a dye infused and the duration for which the dye appeared in the milk. Aqueous solutions of the green-turquoise dye were made containing 50, 100, 150, and 200 mg./5 ml. solutions. Five ml. doses of the dye solutions were infused into four separate quarters of a cow. Milk from each quarter was collected at regular milking times until the milk obtained was completely normal in color. It was observed that the milk from the quarter receiving 50 mg. of the dye possessed a turquoise or green color for approximately four milkings; milk from the quarters receiving 100 and 150 mg. of the dye contained color for five to six milkings; and milk from the quarter receiving 200 mg. contained color for seven to eight milkings. Since the recommended doses of 100,000 units of penicillin for mastitis treatment when infused result in the appearance of the antibiotic in milk for about six milkings following the treatment, it was felt that a dose of 100 mg. of the dye per quarter would be suitable to color the milk for the required period, and this was used in the subsequent studies.

#### RESULTS AND DISCUSSION

*Infusion of the Dye Mixed with Penicillin in an Aqueous Medium and Its Appearance in Milk.* Six healthy lactating cows were infused with 5 ml. doses of penicillin and the dye mixture in each quarter. The milk yield of the cows ranged between 17 and 25 lb./day. The doses consisted of 100,000 units of penicillin in aqueous suspension and 100 mg. of the dye. Following the treatment, milk samples

TABLE I  
*Number of Milkings in which Dye and Penicillin Were Secreted in Milk  
 after Intramammary Infusion of Aqueous Suspension*

Animal no.	Milk yield of animals, lb.	Penicillin infused, units	Dye infused, mg.	No. of milkings*	
				Penicillin detected	Dye detected
1	17	100,000	100	3-4	4-5
2	17	100,000	100	3	4
3	25	100,000	100	5-6	5
4	25	100,000	100	5	5
5	20	100,000	100	4	4
6	19	100,000	100	6	6

\* Results for four quarters.

were collected from each quarter and assayed for the antibiotic, and the color intensity was determined visually and photometrically. The results are presented in table I. It shows the number of milkings that contained penicillin and the number of milkings that contained dye that could be observed visually. It may be seen that the dye appeared in the milk for approximately the same number of milkings as did the antibiotic. Penicillin appeared in milk for three to six milkings and the dye for four to six milkings.

Figure 1 shows typical milk samples collected for six milkings (bottles no. 1 to 6, respectively) from a representative treated quarter selected at random. The bottle marked "C" contains regular mixed herd milk sample. Samples no. 1 to 5 show color in the milk, decreasing in intensity, and the sample no. 6, representing the sixth milking, was normal in color and was comparable to the control. There were wide variations in the visibility indexes of the first milkings obtained in different trials. The samples of milk from the first milking, following the treatment, were all of a dark turquoise color, apparently equal in intensity. They had to be diluted 500 to 2000 times, however, with regular milk to dissipate the color or to become comparable to a normal herd milk sample. The first milking possessed therefore a visibility index of 500 to 2000.

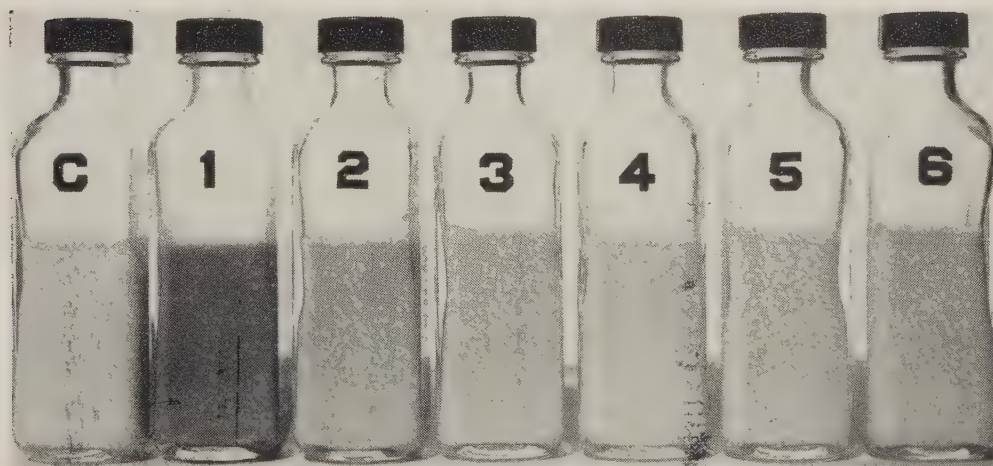


FIG. 1. Milk samples collected for six milkings (numbered 1 to 6, respectively) from a typical quarter following an infusion of penicillin and the dye mixture. The sample "C," a control, is a regular herd milk sample. Samples 1 to 5 show color, decreasing in intensity. Sample 6, representing the sixth milking, was normal in color and comparable to the control.

TABLE II  
Concentrations of Penicillin and Dye Secreted in Milk  
after Intramammary Infusion of Aqueous Suspension\*

No. of milking	Av. penicillin concentration, u./ml.	Visibility index†	Transmittance, %
1	14.00	1800	65
2	0.95	95	92.5
3	0.45	20	95
4	0.11	2	‡
5	0.1	1	‡
6	0.0	0	‡

\* The values presented are averages of four quarters of one representative animal. Essentially similar trend was observed in all the animals.

† Visibility index denotes the number of parts of normal herd milk required per one part of colored milk in order to dissipate the color.

‡ The intensity of the color in the serum after the second or third milking was usually too low to be measured accurately in a spectrophotometer, although the color in the milk could be detected visually.

Table II presents data obtained in one of the representative animals. It shows the concentrations of the antibiotic and the intensities of color, both in terms of visibility indexes and light transmittance, of the milk obtained in several milkings. The values are averages of four quarters. It can be seen that no penicillin could be detected in milk after the fifth milking. There was a direct relationship between the concentration of the antibiotic and the intensity of color in the milk. Also, a direct relationship was observed between the intensity of the color in the milk (visibility index) and the per cent light absorbed by the serum. The results followed essentially the similar trend in all the animals. Although the green color was detectable visually in the milk beyond the second or third milking, the intensity of the color in the serum was too low to measure it accurately in a spectrophotometer, since such milk gave transmittance per cent of more than 95.

*Secretion of Penicillin and the Dye When Infused in an Oil Medium in Contrast to an Aqueous Medium.* Several workers<sup>9,10</sup> have shown that when infused in an oil base an antibiotic appeared in milk for a longer time than when it was infused as a water solution. Consideration was given therefore to comparing the secretion of the dye when infused with penicillin in an oil medium as opposed to an aqueous medium. For these trials, 100 mg. of the dye were mixed with 900,000 units of penicillin in oil and in water suspensions and were infused into each of the four quarters. Two to three different animals were used for each of the suspensions. The results obtained with each suspension were averaged and are presented in table III. It may be seen that when infused in an oil base, the antibiotic persisted in milk for 12 milkings and the color for 11 milkings. In contrast, when the infusion consisted of an aqueous mixture the antibiotic appeared in milk for eight milkings and the dye for 10 milkings. It will be recalled that the penicillin concentrations used in this phase are higher than those in the aqueous infusions mentioned in the first phase of this study.

In the case of the quarters treated with aqueous suspension, a high proportion of the antibiotic secreted was produced in the first two milkings, and the antibiotic secretion fell off very sharply after the second milking. In the case of the quarters infused with the oil suspension the proportion of the antibiotic secreted was not as great in the first two milkings, and the secretion of the antibiotic fell off more gradually. It was interesting to note that the color of the milk obtained during the first

TABLE III  
*Secretion of Penicillin and Dye after Intramammary Infusions  
of Oil and Water Suspensions\**

Milking no.	Oily mixture		Aqueous mixture	
	Penicillin concentration, u./ml.	Index of visibility	Penicillin concentration, u./ml.	Index of visibility
1	77.0	500	230.0	950
2	85.0	320	170.0	550
3	24.5	200	14.0	275
4	17.2	45	3.1	110
5	7.5	18	0.6	40
6	4.7	8	0.25	12
7	1.15	2.5	0.1	6
8	2.1	1.5	0.1	4
9	0.75	1.0	0.0	2.5
10	0.10	1.0	0.0	1.5
11	0.1	0.5	0.0	0
12	0.1	0	—	—
13	0.0	0	—	—

\* The infusion consisted of 900,000 units of penicillin and 100 mg. of the dye per quarter.

two milkings from the animals treated with the aqueous suspension was considerably more intense than that of the milk from the animal receiving the oil suspension.

*Effect of Antibiotic to Dye Ratios upon the Duration of the Color Appearing in Milk.* When a dose consisted of 100 mg. of dye mixed with 100,000 units of penicillin, the dye and the antibiotic appeared in milk for three to six milkings. However, when the same quantity of the dye was mixed with a larger amount of antibiotic, the milk contained color for a longer time and persisted in milk for almost as long as did the antibiotic. Studies were conducted therefore to determine the relationship between concentration of the antibiotic (mixed with a constant amount of the dye) and the duration for which the dye appeared in the milk. Three concentration levels of aqueous penicillin suspension, 100,000, 400,000, or 900,000 units, were used. In each case, the dye concentration was held constant at 100 mg./dose. The dye and the antibiotic were mixed together before administration. The different antibiotic and dye mixtures (consisting of different penicillin levels) were administered to different animals. The results are presented in table IV. The higher the concentration of the antibiotic infused, the longer the time for which it appeared in milk. In most cases, the dye appeared in milk and could be detected visually for almost the same length of time for which the antibiotic appeared in milk. In a few cases, however, the dye appeared in milk for one to two more or less milkings than did the penicillin. On the basis of these results, it is felt that the appearance of the dye in milk is related

TABLE IV  
*Secretion of Penicillin and Dye in Milk Following Intramammary Infusions  
of Different Penicillin and Dye Mixtures*

Mixture no.	Antibiotic concentration per quarter, units	Dye concentration per quarter, units	Ratio*	No. of milkings penicillin detected	No. of milkings dye detected
I	900,000	100	9 : 1	6-9	8-10
II	400,000	100	4 : 1	6-7	6- 6
III	100,000	100	1 : 1	3-6	4- 6

\* For the sake of brevity a mixture of 100,000 units of penicillin and 100 mg. of the dye is considered to have a ratio of 1 : 1.

TABLE V  
*Penicillin and Dye Concentrations Secreted in Milk  
Following Infusion in Only One Quarter*

Milking no.	Right front		Right rear		Left front		Left rear	
	Penicillin concentration, u./ml.	Color visi- bility index	Penicillin concentration, u./ml.	Color visi- bility index	Penicillin concentration, u./ml.	Color visi- bility index	Penicillin concentration, u./ml.	Color visi- bility index
1	37.5	2000	0	0	0	0	0	0
2	2.7	60	0	0	0	0	0	0
3	0.3	20	0	0	0	0	0	0
4	0.1	6	0	0	0	0	0	0
5	0.0	0	0	0	0	0	0	0

to the secretion of the antibiotic in milk. It is felt that when the dye is mixed with the antibiotic the two compounds form a sort of complex and are secreted jointly. This possibility seems to be further substantiated by the fact that there was a direct relationship between the concentration of the antibiotic secreted and the color visibility index.

*Diffusion of Penicillin and the Dye from One Quarter to Another.* Often in mastitis therapy only the infected quarters are treated. Studies were made to determine if an antibiotic and the dye, when infused in one quarter, would be found in the milk from the other quarters. Using the same technique, 100,000 units of penicillin mixed with 100 mg. of the dye were infused only in the right front quarter of an animal. Milk samples were collected from all the four quarters and assayed. The findings relative to the penicillin concentrations and visibility indexes are presented in table V. It was observed that milk from only the infused quarter contained both the antibiotic and the dye, indicating that neither the drug nor the dye diffused from one quarter to another. The findings relative to the fact that the antibiotic did not diffuse from one quarter to another are in agreeemnt with the observations of Randall et al<sup>9</sup> and Hansen et al.<sup>11</sup>

During the course of these studies, the infusion of the dye, with or without the antibiotic, revealed no apparent adverse effect upon the milk yield of the animals. Nor did it cause any visible or noticeable irritation to the udder tissues. It may be pointed out that the dye used in this work was of a technical grade and not approved for human consumption. It is of evident importance that a dye should be nontoxic to human beings if it were to be used in veterinary preparations for the purpose of detecting antibiotics in milk. Feeding trials with animals would be desirable in order to determine whether any toxic effects are produced upon the ingestion of colored milk.

While these experiments were in progress, two other green dyes, Calcocid green SB and Edicol supra green BS, comparable to the green-turquoise dye used in this study, were obtained and tested for use in the detection of antibiotics in milk. Preliminary trials have revealed that both the dyes could be used for the purpose. Additional trials are being continued and the results will be presented elsewhere.

#### SUMMARY

A green-turquoise dye mixed with a penicillin-in-water or penicillin-in-oil preparation and used in intramammary infusions seems to provide a satisfactory method for the visual detection of the antibiotic in milk. The dye appeared in milk,

coloring it turquoise or green, for almost the same length of time as the penicillin appeared in milk. When an antibiotic preparation containing 100,000 units penicillin and 100 mg. of the dye was used, the milk possessed a green color for four to six milkings. The first milking after the treatment contained enough dye to show color even after it was diluted 500 to 2000 times its volume with normal milk. The color intensity was measured in terms of a "visibility index" (number parts of normal milk required to dissipate color in one part of colored milk) and in terms of light absorbence. The index of visibility and light absorbence, which decreased with successive milking, showed a direct relationship with the concentration of penicillin in milk.

Keeping the dye concentration constant, when the infusion contained a larger dose of penicillin, both the antibiotic and the dye appeared in milk for a longer time. It is felt that the dye forms a sort of complex with the antibiotic. The dye infused with penicillin-in-oil preparation appeared in milk for a slightly longer time than when it was infused with a penicillin in water preparation. The dye and the antibiotic did not diffuse from one quarter to another. The infusion of the dye did not cause any apparent irritation to the udder tissues or adverse effect upon the milk yield of the animals.

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#### ADDENDUM

Since the presentation of this paper, an article by Smitasiri et al<sup>12</sup> relative to the use of annatto, Baker's red food dye, and chlorophyll for the detection of antibiotics in milk has appeared in the literature.

#### BIBLIOGRAPHY

1. WELCH, H.; JESTER, W. R., AND BURTON, J. M.: Antibiotics in fluid market milk. Third national survey, *Antib. & Chemo.* 6:369, 1956.
2. WELCH, H.; JESTER, W. R., AND BURTON, J. M.: Antibiotics in fluid milk, *Antib. & Chemo.* 5:571, 1955.
3. SHAHANI, K. M.; GOULD, I. A.; WEISER, H. H., AND SLATTER, W. L.: Observations on antibiotics in a market milk supply and the effect of certain antibiotics on the keeping quality of milk, *Antib. & Chemo.* 6:544, 1956.
4. KOSIKOWSKY, F. V.; HENNINGSON, R. W., AND SILVERMAN, G. J.: The incidence of antibiotics, sulfa drugs and quaternary ammonium compounds in the fluid milk supply of New York State, *J. Dairy Sc.* 34:533, 1952.
5. SHAHANI, K. M., AND BADAMI, M. C.: A resazurin disk assay method for detecting antibiotics and natural starter inhibitory activity in milk, *J. Dairy Sc.* In press.
6. NEAL, C. E., AND CALBERT, H. E.: The Use of 2, 3, 5-triphenyltetrazolium chloride as a test for antibiotic substances in milk, *J. Dairy Sc.* 38:629, 1955.
7. DALGAARD-MIKKELSEN, SV., AND RASMUSSEN, F.: Robefarve i Antibioti kaholdige intramammaria. Forelobig meddilelse, *Nord. Vet.-med.* 9:852, 1957.
8. HARGROVE, R. E.; LEHMAN, R. J., AND MATTHEWS, C. A.: Use of fluorescent materials for the indirect detection of antibiotics in milk, *J. Dairy Sc.* 41:617, 1958.
9. RANDALL, W. A.; DURBIN, C. G.; WILNER, J., AND COLLINS, J. H.: Antibiotic concentration and duration in animal tissue and body fluids. I. Blood serum and milk of cows. *In: Antibiotics Annual 1953-1954*, New York, Medical Encyclopedia, Inc., 1954, p. 421.
10. JACKSON, W. F., AND BRYAN, C. S.: Penicillin milk levels in cows following intramammary administration, *Vet. Med.* 45:395, 1950.
11. HANSEN, H. C.; WIGGINS, G. E., AND BOYD, J. C.: Modern methods of mastitis treatment cause trouble in the manufacture of fermented dairy products, *J. Milk & Food Technol.* 13:359, 1950.
12. SMITASIRI, T.; KOSIKOWSKI, F. V.; GUTHRIE, R. S., AND FINCHER, M. G.: Dyes as markers for antibiotic-contaminated milks, *J. Milk and Food Technol.* 21:255, 1958.

# Factors Affecting the Loss of Antibiotic Activity in Milk

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Several investigators have reported the partial inactivation of antibiotics after the addition of raw milk and subsequent additional losses of antibacterial activity that occurred during cold storage.<sup>2,5,6</sup> Shahani et al.<sup>4,6</sup> reported losses during seven days' storage at 4 C. of 37 per cent for penicillin, 21 per cent for streptomycin, and 15 per cent for chlortetracycline.

The cause of the losses of antibiotic activity in milk has not been elucidated. Shahani<sup>4</sup> suggested that bacterial activity of milk enzymes activity might be responsible for the loss of antibacterial activity. Jepsen and Overby<sup>2</sup> advanced a theory that interaction of antibiotic with some milk constituents might explain the loss of penicillin during storage of milk. In blood loss of antibiotic activity of penicillin was related to interaction with serum albumin by Tompsett et al.<sup>8</sup> Recently, Weinberg<sup>9</sup> reported that divalent ions reduced tetracycline activity in blood.

This investigation was undertaken to determine the cause or causes for loss of antibiotic activity in milk, with particular attention being directed toward the role of proteins and calcium in antibiotic inactivation.

## PROCEDURE

The antibacterial activity of the antibiotics was measured by the disc assay method of Silverman and Kosikowski<sup>7</sup> as modified by Shahani et al.<sup>5</sup> Whey agar (Difco) was used as the medium for the assay of penicillin, and oxytetracycline enriched assay agar (Difco) was used for the assay of chlortetracycline and oxytetracycline. Spore suspensions of *Bacillus subtilis* were used as the test organisms in the assay of penicillin, whereas, *Bacillus cereus* spore suspensions were used in the assay of the tetracyclines. Ten ml. portions of seeded agar were pipetted into sterile 100 ml. diameter flat bottom dishes. A sterile one-half inch filter paper disc (SMS no. 740-E) was used. A standard curve was prepared for each assay. Dialysis equilibrium as suggested by Davis<sup>1</sup> was utilized as a means of studying antibiotic interaction with milk constituents.

The milk product or constituent to be tested for interaction with antibiotic was dialyzed in a Viscing dialyzing membrane for 48 hours at 5 C. using a phosphate buffer at pH 6.6 and an ionic strength of 0.10 with 80 times as much buffer as milk or milk protein solutions. Another dialysis membrane containing 50 ml. of the buffer was dialyzed against the same buffer to serve as a control. After 48 hours, the milk product and control membranes were removed and dialyzed separately against 1000 ml. of buffer containing 0.5 units/ml. of penicillin. After the second dialysis, which lasted 48 hours also, the contents of both sacs containing buffer and milk products and the respective dialysates were assayed for antibiotic activity by the disc assay procedure. Analysis of the dialyzing membrane was made and it was found that no absorption of the penicillin occurred in the membrane, whereas, adsorption of oxytetracycline and chlortetracycline occurred. However,

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TABLE I

*Comparison of the Inactivation of Penicillin and Chlortetracycline in Skim Milk, Raw Whey, Pasteurized Whey, and Distilled Water*

Sample	Antibiotic added	Degree of immediate inactivation, %		Degree of inactivation after 2 days storage at 4 C., %		Total loss, %	
		Range	Average	Range	Average	Range	Average
Raw whey	Penicillin	0-8	3.3	32-60	46.0	32-66	49.3*
Pasteurized whey	Penicillin	0-12	4.0	0-25	16.1	12-25	19.1
Raw skim	Penicillin	0-10	5.8	10-62.5	44.3	20-72.5	48.1
Pasteurized skim	Penicillin	0-11	3.4	0-27.6	18.2	10-37	20.1
Water	Penicillin	0-0	0.0	16-21.5	19.3	16-21.5	19.3
Raw whey	Chlortetracycline	0-3.3	5.0	6.3-21.1	11.2	6.3-29.9	14.2
Pasteurized whey	Chlortetracycline	0-4.2	2.0	4.2-22.1	13.2	4.2-24.0	15.2
Raw skim	Chlortetracycline	29.2-66.0	54.4	0-11.6	7.6	45.9-79.6	61.1
Pasturized skim	Chlortetracycline	35.6-65.7	49.6	0-8.9	5.6	36.5-58.5	55.2
Water	Chlortetracycline	0-4.2	2.0	0-4.2	0.6	0-4.6	2.6

\* Average of two trials due to no growth of *B. subtilis*. All other figures reported are the average of three trials with each antibiotic.

the amount adsorbed was the same when dialysis was conducted against buffer or against a milk product. Therefore, no correction for membrane adsorption was made. Interaction was measured by comparing the ratio of the antibiotic concentration ( $\mu$ /ml.) inside the dialysis sac to the concentration ( $\mu$ /ml.) in the dialysate. Perfect equilibrium is equivalent to a ratio of 1.0, whereas, concentration of the antibiotic inside the dialysis sac increased the ratio to a value greater than 1.0.

Measurement of radioisotopic activity, with  $C^{14}$  labeled antibiotics, was made with a nuclear (Chicago) model 181A scaler and a windowless gas-flow detector. All data were corrected for self-absorption. Samples were counted for sufficient time to provide a standard error of less than 2.0 per cent.

## RESULTS

*Comparison of Loss of Activity of Penicillin, Chlortetracycline, and Oxytetracycline in Skim Milk, Whey, and Water.* Small amounts of penicillin G (0.5) chlortetracycline (0.5), and oxytetracycline (0.5) were added to raw and pasteurized skim milk and whey and to sterile distilled water. The whey was obtained from the skim milk by rennet coagulation and centrifugation. Analyses were made immediately after the antibiotic was added to the milk and after 48 hours' storage at 4 C. Since the results for the two tetracyclines were the same, only the data for the penicillin and chlortetracycline trials are tabulated in table I.

Relatively low decreases in penicillin activity were observed upon immediate analysis of either whey or milk. However, upon storage for 48 hours at 4 C., the amount of inactivation in raw whey and raw skim milk increased greatly. Considerable variation was observed between trials. The average loss immediately after the addition of raw skim milk was 5.8 per cent, with the loss increasing to 48.1 per cent after storage. The loss in raw whey was similar. The loss of activity in pasteurized skim milk and whey was about the same as in distilled water. These results showed that the cause of major loss in penicillin activity occurred during storage and was related to the raw product. The bacterial counts were between 10,000 to 20,000/ml. and could not be related to the storage loss.

TABLE II  
Effect of Equilibrium Dialysis on the Concentration of Penicillin in  
Skim Milk, Whey, and Buffer

Trial	Total concentration of penicillin after 48 hours dialysis against buffer*				Ratio of units/ml. in dialysis sac to units/ml. in dialysate			
	Buffer	Skim milk	Buffer	Whey	Buffer	Skim milk	Buffer	Whey
1	400	400	482	480	0.96	.97	.96	.97
2	580	640	484	480	0.92	.78	1.04	1.1
3	480	500	623	576	1.04	.96	0.87	0.67
4	520	580	577	476	1.03	.76	.99	0.93

\* Fifty ml. samples dialyzed against 1000 ml. of phosphate buffer, pH 6.6,  $\tau/2$  0.10.

The major loss of chlortetracycline occurred immediately after its addition to milk, either raw or pasteurized, and was associated with the rennet coagulable fraction of milk. An average loss of more than 50 per cent was observed immediately after the addition of chlortetracycline to either raw or pasteurized skim milk, whereas in raw and pasteurized whey losses ranged from 2 to 5 per cent. Subsequent loss during storage at 4 C. was about 10 to 15 per cent in both raw and pasteurized products.

*Penicillin Activity in Milk and Whey after Equilibrium Dialysis.* Pasteurized skim milk and whey were utilized in equilibrium dialysis along with buffer controls to study inactivation and interaction. Pasteurized products were used to eliminate storage losses associated with raw milk and whey. The results for four trials are summarized in table II.

As would be expected, very little difference in antibiotic activity was evident between the dialyzed milk and buffer control. Slightly less antibiotic diffused through the membrane containing milk than through the membrane containing buffer. No loss of antibiotic activity occurred. With whey no definite relationship could be established between the whey proteins and penicillin activity after equilibrium dialysis. Two of the trials showed greater total penicillin in the whey than in the buffer controls, whereas, the other two trials indicated the reverse effect. No evidence of interaction could be observed.

*Penicillin Activity in  $\alpha$ -Casein and  $\beta$ -Lactoglobulin after Equilibrium Dialysis.* Although no interaction and no inactivation could be ascertained on equilibrium dialysis of penicillin with dialyzed milk or whey, further trials were conducted with two purified milk proteins,  $\alpha$ -casein and  $\beta$ -lactoglobulin. Two per cent solutions of sodium- $\alpha$ -caseinate and  $\beta$ -lactoglobulin were dialyzed for 48 hours against the phosphate buffer and equilibrium dialysis experiments were conducted as previously outlined. The data are presented in table III.

TABLE III  
Effect of Equilibrium Dialysis on the Concentration of Penicillin  
in  $\alpha$ -Casein,  $\beta$ -Lactoglobulin and Buffer

Trial	Total concentration of penicillin		Ratio $\gamma$ /ml. in dialysis sac to $\gamma$ /ml. in dialysate		Total concentration of penicillin		Ratio $\gamma$ /ml. in dialysis sac to $\gamma$ /ml. in dialysate	
	Buffer	$\alpha$ -Casein	Buffer	$\alpha$ -Casein	Buffer	$\beta$ -Lactoglobulin	Buffer	$\beta$ -Lactoglobulin
1	630	660	1.0	0.78	609	614	1.06	0.82
2	728	681	0.81	0.66	400	417	1.10	0.86
3	526	506	1.05	0.66	—	—	—	—
4	566	530	0.98	0.77	—	—	—	—

TABLE IV  
*Effect of Age of  $\alpha$ -Casein in Equilibrium Dialysis of C<sup>14</sup> Penicillin  
in  $\alpha$ -Casein and Buffer\**

Age of casein, days	C <sup>14</sup> penicillin activity after 48 hours dialysis against buffer†						C <sup>14</sup> penicillin activity after 48 hours dialysis against $\alpha$ -casein					
	Dialyzed buffer			Dialysate			Dialyzed $\alpha$ -casein			Dialysate		
	u./ml.	cpm/ml.	cpm/u.	u./ml.	cpm/ml.	cpm/u.	u./ml.	cpm/ml.	cpm/u.	u./ml.	cpm/ml.	cpm/u.
0	0.68	44	65	0.63	52	82	0.30	100	333	0.66	64	97
7	0.66	80	121	0.52	74	142	0.13	126	969	0.56	78	139
14	0.66	102	155	0.46	106	230	0.05	158	3160	0.62	96	158

\* 0.5 units of C<sup>14</sup> penicillin was added per ml. of buffer.

† Ten ml. of buffer and casein samples dialyzed against 200 ml. of phosphate buffer, pH 6.6, r/2 0.10.

The penicillin activity was consistently less in the dialyzed  $\alpha$ -casein than in the buffer controls and the total concentration in the  $\alpha$ -casein trials was less than in the corresponding controls. The data show inactivation but no evidence of interaction could be obtained.

$\beta$ -Lactoglobulin exerted no inactivation effect on penicillin and the amount of penicillin diffusing into the dialysis sac was the same for both the  $\beta$ -lactoglobulin and the buffer control.

*Effect of Aging of  $\alpha$ -Casein on Activity of C<sup>14</sup> Penicillin after Equilibrium Dialysis.* The fact that  $\alpha$ -casein apparently caused an inactivation of penicillin was studied further using C<sup>14</sup> penicillin and equilibrium dialysis.  $\alpha$ -Casein that had been freshly prepared and the same protein solution after aging for 7 to 15 days prior to equilibrium dialysis trials were used. Analysis for penicillin activity was made by the disc assay and the C<sup>14</sup> level was determined using a nuclear (Chicago) model 181A scaler and windowless gas-flow detector. The initial specific activity of the C<sup>14</sup> in the penicillin was 150 count/min. (cpm) per unit. The results are presented in table IV.

With freshly prepared casein the data were similar to those found with penicillin and  $\alpha$ -casein. A loss in the total activity was observed in the  $\alpha$ -casein as compared to the buffer control. The penicillin activity in the dialysate was about one-half that in the dialyzed buffer control, whereas the C<sup>14</sup> in the dialyzed  $\alpha$ -casein was almost twice that in the buffer control. All of the loss of penicillin activity could be accounted for on the basis of the C<sup>14</sup> in the dialysed  $\alpha$ -casein solution.

As the casein aged, the loss of penicillin activity increased markedly and the amount of active penicillin also was greatly decreased. The amount of C<sup>14</sup> within the dialysis sac containing  $\alpha$ -casein, however, remained about the same.

Redialysis of the 14 day old  $\alpha$ -casein solution containing penicillin against dis-

TABLE V  
*Effect of Equilibrium Dialysis on the Concentration of Chlortetracycline  
in  $\alpha$ -Casein,  $\beta$ -Lactoglobulin, and Buffer*

Trial	Total antibiotic		Ratio $\gamma$ /ml. in dialysis sac to $\gamma$ /ml. in dialysate		Total antibiotic		Ratio $\gamma$ /ml. in dialysis sac to $\gamma$ /ml. in dialysate	
	Buffer	$\alpha$ -Casein	Buffer	$\alpha$ -Casein	Buffer	$\beta$ -Lactoglobulin	Buffer	$\beta$ -Lactoglobulin
1	502	540	.92	1.56	420	460	0.98	1.02
2	637	580	.92	1.73	430	460	0.98	0.98
3	511	527	.92	1.85	—	—	—	—
4	490	488	.72	1.65	—	—	—	—

TABLE VI  
Effect of Equilibrium Dialysis on the Concentration of  
Chlortetracycline in Skim Milk, Whey, and Buffer

Trial no.	Concentration of chlortetracycline after 48 hours' dialysis against, $\gamma$				Ratio of $\gamma$ /ml. in dialyzing sac to $\gamma$ /ml. in dialysate			
	Buffer	Milk	Buffer	Whey	Buffer	Milk	Buffer	Whey
1	399	260	454	356	1.50	1.66	0.90	0.95
2	627	545	522	535	0.91	1.06	0.91	1.0
3	604	548	523	521	0.85	1.10	0.75	0.95

tilled water for 48 hours resulted in diffusion of only 1.1 per cent of the  $C^{14}$  of the penicillin out of the  $\alpha$ -casein.

*Chlortetracycline Activity in  $\alpha$ -Casein and  $\beta$ -Lactoglobulin after Equilibrium Dialysis.* The milk proteins,  $\alpha$ -casein and  $\beta$ -lactoglobulin, were utilized in the same manner as for penicillin. The results of four trials are summarized in table V. With  $\alpha$ -casein no inactivation of chlortetracycline activity was observed, but interaction occurred as evidenced with the marked increase in antibiotic activity in the dialyzed  $\alpha$ -casein as compared to the buffer control. The data indicated the  $\alpha$ -casein does not contribute to the inactivation of chlortetracycline in dialyzed skim milk. Neither inactivation nor interaction was observed in dialysis equilibrium trials with  $\beta$ -lactoglobulin. Nearly identical results were also obtained with oxytetracycline.

*Chlortetracycline and Oxytetracycline Activity in Milk and Whey after Equilibrium Dialysis.* The data for four equilibrium dialysis trials are summarized in table VI. The total chlortetracycline activity in dialyzed milk was consistently less than in the dialyzed buffer control. The higher concentration of the chlortetracycline inside the dialyzed milk sac as compared to the buffer indicated possible interaction. However, the data show no evidence of inactivation or interaction of the whey proteins in respect to chlortetracycline. The same results were obtained with oxytetracycline.

*Calcium  $\alpha$ -Casein Interrelationship in the Inactivation of Chlortetracycline and Oxytetracycline.* The fact that  $\alpha$ -casein interacted with the tetracyclines but caused no inactivation suggested that the calcium, which was associated with the casein in dialyzed skim milk, might be the cause of inactivation. In these trials  $C^{14}$  labeled oxytetracycline was used as the antibiotic. Dialysis equilibrium trials were conducted with the buffer alone, buffer with 300 mM of calcium chloride added,  $\alpha$ -casein alone and  $\alpha$ -casein with 300 mM of calcium chloride added. The results are presented in table VII.

TABLE VII  
Effect of the Addition of Calcium Chloride on  $C^{14}$  Oxytetracycline Activity  
in  $\alpha$ -Casein after Equilibrium Dialysis

Sample	Total antibiotic after dialysis	$C^{14}$ oxytetracycline activity					
		Dialyzed samples			Dialysate		
		$\gamma$ /ml.	cpm/ml.	cpm/u.	$\gamma$ /ml.	cpm/ml.	cpm/u.
Buffer control	480	.41	87	216	.46	110	237
Buffer + 300 mM calcium chloride	482	.45	102	227	.46	96	209
$\alpha$ -Casein	476	.96	187	207	.43	95	226
$\alpha$ -Casein + 300 mM calcium chloride	428	.56	152	271	.40	88	220

Calcium chloride alone had no effect on the activity of  $C^{14}$  distribution of the oxytetracycline after dialysis. On the other hand, the presence of calcium ions together with  $\alpha$ -casein resulted in inactivation of the oxytetracycline and a slight apparent decrease in the interaction of  $\alpha$ -casein and the oxytetracycline. The same experiments conducted with unlabeled chlortetracycline revealed similar results in respect to antibiotic activity, but no indication of the carbon distribution could be obtained.

*Some Properties of the  $\alpha$ -Casein Complex with Tetracyclines.* To determine the possible reversibility of the calcium-casein complex with oxytetracycline and chlortetracycline, the dialyzed solutions were redialyzed for 48 hours, against distilled water. The tetracyclines in the buffer control membranes readily redialyzed. However, with  $C^{14}$  oxytetracycline in  $\alpha$ -casein-calcium, neither antibiotic activity nor  $C^{14}$  could be detected in the dialysate, and all of the activity and  $C^{14}$  could be detected inside the dialysis membrane. With chlortetracycline, an average of 9.6 per cent of the antibiotic diffused from  $\alpha$ -casein into the dialysate.

Several of the  $\alpha$ -casein samples containing  $C^{14}$  oxytetracycline were precipitated with 1 *N* hydrochloric acid, centrifuged, washed with distilled water, and the whey and washings filtered. Only 4.7 to 8.6 per cent of the  $C^{14}$  could be recovered in the acid filtrates.

An attempt was made to use paper electrophoresis to study the complex but it was unsuccessful because of the severe tailing of  $\alpha$ -casein which corresponded to the area of migration of the tetracycline.

#### DISCUSSION

No direct evidence was obtained to determine whether bacteria or milk enzymes were responsible for the loss of penicillin during storage. Various enzymes other than penicillin have been reported to inactivate penicillin. These enzymes included clarase and take-diasase.<sup>3</sup> Therefore, it is conceivable that the loss of penicillin activity in milk might be caused by the action of one or more enzymes that might possibly be present in small quantities in the raw product. Another explanation, suggested by the effect of aging of  $\alpha$ -casein on penicillin inactivation, would be that slow decomposition of milk proteins with an increase in free amino groups could interact with penicillin causing its inactivation.

Since the major loss of activity of the tetracycline antibiotics occurred immediately upon their addition to milk, it is not probable that enzymatic action is responsible for the large immediate loss observed with these antibiotics. Enzymatic action could be responsible for the small storage loss that occurred, even though the storage loss of the tetracyclines was about the same in raw and in pasteurized products. Based on the results of this study, the immediate and storage loss of antibacterial activity of these antibiotics would seem to be entirely separate. Since various workers have found that the activity of certain antibiotics is suppressed by blood serum proteins,<sup>8</sup> the most logical assumption would seem to be that either the milk proteins in general, or possibly some specific fraction of the milk proteins, are the milk constituents responsible for the immediate decrease in the activity of the tetracycline antibiotic in milk. The view was supported by the results obtained in this study. The fact that no loss was caused by dialysis against whey indicated that the casein system was primarily associated with the inactivation. This was further supported by the fact that no interaction or inactivation occurred in the presence of the major whey protein,  $\beta$ -lactoglobulin. This would agree with work

with blood proteins in that globulins were found to be unable to interact with penicillin.<sup>6</sup>

The dialysis equilibrium experiments using  $\alpha$ -casein with chlortetracycline and C<sup>12</sup> and C<sup>14</sup> oxytetracycline clearly show definite interaction between these tetracyclines and the  $\alpha$ -casein. The validity of using dialysis equilibrium as an index of protein interaction has been established by several investigators. Although interaction was obtained, the absence of inactivation after dialysis in the presence of  $\alpha$ -casein revealed that some other component of the dialyzed milk is involved in tetracycline inactivation. This could be another constituent of the casein system or a mineral ion as suggested by Weinberg.<sup>9</sup> However, calcium ions alone had no inhibitory effect. The fact that a combination of calcium and  $\alpha$ -casein is essential to produce inactivation provides evidence that the calcium-caseinate system of milk is the active component in inactivating tetracyclines when added to milk. Whether other components of casein or other mineral ions contributed to the inactivation was not determined. This might explain the lack of quantitative agreement in the amount of inactivation that occurred in dialyzed skim milk and in dialyzed  $\alpha$ -casein. Another possible contributing factor could be that sodium- $\alpha$ -caseinate was used as the starting material, and the extent to which it was converted to calcium- $\alpha$ -caseinate would be incomplete.

The nature of the tetracycline- $\alpha$ -casein complex was not determined. The data appeared to suggest a fairly strong binding, since no oxytetracycline and only a small fraction of the chlortetracycline activity diffused from the complex when these solutions were redialyzed against distilled water. This was supported also by the insignificant quantities of the oxytetracycline that was found in the acid filtrate when the oxytetracycline- $\alpha$ -casein complex was precipitated with hydrochloric acid.

#### SUMMARY

The loss of antibiotic activity in milk was found to be markedly different for penicillin and for the tetracyclines, chlortetracycline and oxytetracycline. The loss of penicillin immediately after addition of the antibiotic to skim milk or whey was relatively low, whereas, considerable inactivation of penicillin occurred during storage of either raw milk or whey at refrigeration temperature. Pasteurization of the milk or whey prior to addition of penicillin decreased the loss of penicillin activity during storage. The major loss of oxytetracycline and chlortetracycline activity occurred immediately after addition of these antibiotics to either raw or pasteurized skim milk, with only slight losses occurring during cold storage of the milk to which they had been added. The immediate loss of oxytetracycline and chlortetracycline activity was much less in whey than in skim milk, although considerable losses occurred when the whey containing those antibiotics was stored at refrigeration temperatures.

Equilibrium dialysis experiments indicated that no interaction occurred between penicillin and either the skim milk or whey protein system. Neither was the activity in the antibiotic affected greatly by the skim milk or the whey. However, interaction was observed between chlortetracycline and the protein system of both skim milk and whey. In skim milk the interaction was accompanied by a considerable amount of inactivation in the antibiotic. No interaction or inactivation occurred in the whey system.

Equilibrium dialysis experiments with purified milk proteins indicated no obvious interaction of penicillin with  $\alpha$ -casein. Neither did  $\alpha$ -casein cause an appreci-

able decrease in the activity of the antibiotic. Considerable interaction of chlortetracycline with  $\alpha$ -casein was observed, although this interaction was not accompanied by a decrease in the antibacterial potency of the antibiotic.

Radioactive tracer studies revealed that the antibacterial activity of penicillin was decreased considerably by  $\alpha$ -casein, and the degree of inactivation seemed to be directly related to the age of the  $\alpha$ -casein solutions. There was an indication also that the inactivated penicillin remained adsorbed on the  $\alpha$ -casein, based on the  $C^{14}$  distribution.

Radioactive oxytetracycline interacted considerably with the  $\alpha$ -casein, but no inactivation of oxytetracycline appeared to be associated with this interaction. Calcium ions in the absence of  $\alpha$ -casein had no effect on the activity of this antibiotic. Calcium ions in combination with  $\alpha$ -casein caused considerable inactivation of both oxytetracycline and chlortetracycline. The inactivation of oxytetracycline by the  $\alpha$ -casein-calcium solution was accompanied by subsequent decreases in the oxytetracycline protein interaction, whereas this was not observed in the case of chlortetracycline.

The oxytetracycline  $\alpha$ -casein complex was not reversible, whereas the complex formed by chlortetracycline and  $\alpha$ -casein was reversible to a slight extent. When the oxytetracycline  $\alpha$ -casein was precipitated with hydrochloric acid, only a small part of the oxytetracycline activity was present in the acid filtrate.

Purified  $\beta$ -lactoglobulin had no significant effect on either penicillin or chlortetracycline activity, and no chemical interaction occurred between this whey protein and either of these antibiotics.

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#### BIBLIOGRAPHY

1. DAVIS, B. D.: Binding of sulfonamides by plasma proteins, *Science* 95:78, 1942.
2. JEPSEN, A., AND OVERBY, A. J.: The disturbing effects of penicillin in milk, *Neth. Milk and Dairy J.* 5:157, 1951.
3. LAWRENCE, C. A.: Effects of enzyme preparations upon penicillin. I. A method for testing penicillin for sterility, *J. Bact.* 49:47, 1945.
4. SHAHANI, K. M.: Factors effecting Terramycin activity in milk, broth, buffer and water, *J. Dairy Sci.* 42:382, 1958.
5. SHAHANI, K. M.; GOULD, I. A.; WEISER, H. H., AND SLATTER, W. L.: Effect of heat treatments on streptomycin and chlortetracycline in milk. *In: Antibiotics Annual 1954-1955.* New York. Medical Encyclopedia, Inc., 1954; p. 353.
6. SHAHANI, K. M.; GOULD, I. A.; WEISER, H. H., AND SLATTER, W. L.: Stability of small concentrations of penicillin in milk as effected by heat treatment and storage, *J. Dairy Sc.* 39:971, 1956.
7. SILVERMAN, G. J., AND KOSIKOWSKI, F. V.: Systematic testing of inhibitory substances in milk, *J. Milk and Food Tech.* 15:120, 1952.
8. TOMPSETT, R.; SHULTZ, S., AND McDERMOTT, W.: The relation of protein binding to the pharmacology and antibacterial activity of penicillins X, G, dihydro F, and K, *J. Bact.* 53:581, 1947.
9. WEINBERG, E. P.: 58th General Meeting, Society American Bact. Chicago, 1958.

# Nystatin in the Control of Fungal Infections of Orchids

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The flower-producing industry suffers a loss of millions of dollars annually due to fungal diseases of plants and cut flowers. Particularly susceptible to fungal spoilage are China asters, chrysanthemums, carnations, gladioli, lilies, gardenias, narcissuses, roses, and orchids. The magnitude of the problem can best be demonstrated by citing some of the commercial flower crop losses as reported by the United States Department of Agriculture<sup>1</sup> in the year 1954. For example, the yearly losses primarily due to fungal diseases are 20 per cent of the gladioli, 6 per cent of the lilies, 11 per cent of the carnations, and 12 per cent of the roses grown. The loss to the producers for these flowers alone is in excess of 12 million dollars per year.

In view of the availability and high antimicrobial activity of antibiotics, it was reasonable to consider the application of a suitable agent for the control of fungal diseases of plants. One antifungal agent that is readily available in large quantities is nystatin (Mycostatin\*). Therefore, we investigated the effects of the application of nystatin in the control of fungal spotting of orchids, flowers of high economic value easily susceptible to fungal attack.

## MATERIALS AND METHODS

The flowers used in these studies were hybrid orchids of the *Cattleya* family. Six to 10 orchids were used for a given experimental condition, and, whenever possible, control and test flowers were taken from the same stem. In the usual case, the flowers were kept in water in rubber-capped vials.

The fungi isolated from flowers were grown on potato dextrose agar incubated at 25°C. for 10 days. Spore suspensions required for use in experimental infections were made by washing the growth off slants with sterile water containing 0.01 per cent Dupanol C. The resulting heavy suspension of spores was sprayed on flowers with a heat-sterilized DeVilbiss atomizer.

Solutions of nystatin were prepared by dissolving the crystalline material in dimethylsulfoxide, usually at a concentration of 20 mg./ml. These were further diluted with distilled water to yield finely dispersed suspensions. The usual concentration of nystatin in suspensions sprayed on flowers was 300 units/ml.† Approximately 2 ml. of suspension was needed to wet the surfaces of each orchid. After spraying, the flowers were dried at room temperature.

The general procedure for studying the spoilage of variously treated orchids was to place them in covered glass tanks or deep aluminum pans lined and sealed with cellophane. High relative humidity was maintained by using water-soaked cotton pads.

To determine the *in vitro* activity of nystatin against a group of phytopathogenic fungi, the agar dilution method was employed. Test organisms were grown at 25°C. for two to three weeks on the following medium: Bacto tryptone, 5 Gm.; malt extract, 3 Gm.; glucose, 10 Gm.; yeast extract, 3 Gm.; distilled water, to 1000 ml.

\* The trade name of E. R. Squibb & Sons Division, Olin Mathieson Chemical Corp., for nystatin is Mycostatin.

† Nystatin used in these studies contained 3000 units/mg.

The resulting growth was washed off with 8 ml. of sterile water containing 0.01 per cent Dupanol C to give a heavy suspension of spores and mycelial fragments or mycelial fragments alone in the case of nonsporulating organisms. For the test, we used Petri plates containing the medium, in which nystatin was incorporated in concentrations decreasing by a factor of 2. The surface of the agar was inoculated with the spore suspension applied with a serological pipette. Control plates containing no antifungal agent were similarly inoculated. In the case of *Pythium* and *Phytophthora* species, 5 mm. squares were cut from the edge of actively growing colonies and placed on the plates containing nystatin. The plates were incubated at 25C. and were observed daily for growth. The minimal inhibiting concentration (MIC) of nystatin for the various fungal species was the lowest concentration that completely inhibited the fungus after a period of incubation adequate for the growth of the control, usually ranging from 48 to 120 hours. Concentrations lower than the MIC frequently permitted only partial growth of the fungi. Although prolonged incubation of the agar plates led to some decrease in the potency of nystatin,<sup>2</sup> the inhibiting concentration, however, was not increased in most cases by more than twofold, rarely fourfold.

#### DISCUSSION AND RESULTS

Fungal spotting of greenhouse orchids is primarily a problem in the shipping and holding of cut flowers, especially when climatic conditions are conducive to fungal growth and during the holiday seasons when packing and storage facilities become overburdened. Warm, humid weather may also cause spoilage of orchids on plants prior to cutting.

The growth of fungi on orchids produces unsightly discolorations, in small areas at first, which become progressively larger. The presence of these spots destroys the market value of the flower. Conditions of temperature and humidity are critical in determining the rate and type of spoilage that occurs.

At first, we attempted to protect naturally infected orchids from petal and sepal spoilage due to fungi by placing the cut stems into suspensions of nystatin at 300 and 30 units/ml. These specimens were subjected to high humidity in glass jars and incubated at 25 and 4C. The degree of spoilage of the nystatin-treated orchids was equivalent to that of the controls. Apparently, an inadequate concentration of the active antifungal agent was transported to the areas involved in the spoilage.

The antifungal effect of nystatin when applied directly to the petals and sepals was then investigated. Flowers naturally contaminated with fungi were sprayed with a nystatin suspension and placed in high humidity at 4C. Control flowers that had been sprayed with sterile water developed brown spots in seven days, and in 14 days they were covered with coalescing areas of decay and mycelial growth, but the nystatin-treated flowers were completely protected from spotting for the same period.

In the course of studying orchids contaminated with fungi indigenous to the plant and greenhouse, it became apparent that there existed considerable variation in the number of fungal colonies occurring on each flower. Therefore, an experimental infection was established that ensured heavy and equal seeding of each flower. The organism used in the experimental infection, a *Botrytis* species, was the one most commonly isolated from naturally infected flowers. A suspension of the *Botrytis* spores sprayed on orchids produced several hundred spots in two days, with sporulation occurring in six days. Other fungi, such as *Alternaria* and *Fusaria*, were isolated, but under the conditions of the experiment (i.e., incubation at 17C. at high

TABLE I  
Activity of Nystatin against Phytopathogenic Fungi

Organism	MIC in units/ml. of agar
<b>Ascomycetes</b>	
<i>Calonectria graminicola</i> ( <i>Fusarium nivale</i> )	1.6 or <
<i>Ceratostomella pilifera</i>	3.1
<i>Ceratostomella ulmi</i>	6.3
<i>Claviceps paspali</i>	6.3
<i>Claviceps purpurea</i>	3.1
<i>Diaporthe conorum</i> ( <i>Phomopsis occulta</i> )	12.5
<i>Endothia parasitica</i>	3.1
<i>Gibberella baccata</i> ( <i>Fusarium lateritum</i> )	6.3
<i>Gibberella zeae</i> ( <i>Fusarium graminearum</i> )	12.5
<i>Glomerella cingulata</i>	1.6 or <
<i>Hypomyces ipomoea</i> ( <i>Fusarium javanicum</i> )	25
<i>Leptosphaeria coniothyrium</i> ( <i>Coniothyrium fuckelii</i> )	3.1
<i>Mycosphaerella pinodes</i> ( <i>Ascochyta pisi</i> )	1.6 or <
<i>Physalospora rhodina</i> ( <i>Diplodia natalensis</i> )	3.1
<i>Sclerotinia fruticola</i>	1.6 or <
<i>Sclerotinia homoeocarpa</i>	1.6 or <
<i>Sclerotinia sclerotiorum</i>	1.6 or <
<b>Basidiomycetes</b>	
<i>Corticium fuciforme</i>	12.5
<i>Fomes annosus</i>	6.3
<i>Lenzites betulinus</i>	6.3
<i>Polyporus galactinus</i>	6.3
<i>Rhizoctonia solani</i>	6.3
<i>Stereum purpureum</i>	6.3
<i>Tilletia horrida</i>	12.5
<i>Typhula graminum</i>	3.1
<i>Ustilago zeae</i>	6.3
<b>Phycomycetes</b>	
<i>Circinella umbellata</i>	1.6 or <
<i>Phytophthora infestans</i>	>200
<i>Phytophthora parasitica</i>	50
<i>Pythium debaryanum</i>	>8000
<i>Pythium ultimum</i>	800
<i>Rhizopus nigricans</i>	12.5
<b>Fungi imperfecti</b>	
<i>Alternaria solani</i>	3.1
<i>Aspergillus niger</i>	12.5
<i>Botrytis allii</i>	3.1
<i>Botrytis cinerea</i>	1.6 or <
<i>Botrytis tulipae</i>	12.5
<i>Botrytis</i> species (orchid isolate)	6.3
<i>Cercospora musae</i>	3.1
<i>Cladosporium fulvum</i>	12.5
<i>Colletotrichum lindemuthianum</i>	12.5
<i>Colletotrichum phomoides</i>	1.6 or <
<i>Colletotrichum pisi</i>	3.1
<i>Cryptostictis caudata</i>	25
<i>Curvularia brachyspora</i>	3.1
<i>Curvularia lunata</i>	6.3
<i>Curvularia maculans</i>	6.3
<i>Cylindrocarpon radicicola</i>	50
<i>Diplodia zeae</i>	1.6 or <
<i>Fusarium oxysporum</i> f. <i>cubense</i>	50
<i>Fusarium oxysporum</i> f. <i>lycopersici</i>	50
<i>Fusarium oxysporum</i> f. <i>narcissi</i>	12.5
<i>Fusarium roseum</i>	12.5
<i>Fusarium solani</i> f. <i>martii</i>	25
<i>Helminthosporium</i> species	3.1
<i>Hormodendrum</i> species	6.3
<i>Penicillium digitatum</i>	12.5
<i>Penicillium expansum</i>	25
<i>Penicillium italicum</i>	25
<i>Pestalotia funerea</i>	1.6 or <
<i>Septoria lycopersici</i>	50
<i>Sphaceloma symphetricarpi</i>	25
<i>Trichothecium roseum</i>	100
<i>Verticillium dahliae</i>	3.1

humidity), we were unable to obtain adequate fungal spoilage with these organisms. Experiments by Feder<sup>3</sup> indicate that temperature of incubation is critical, since he was not able to produce an infection with *Alternaria* at 16 C. after incubation for 12 days.

Nystatin proved highly effective in the control of experimental *Botrytis* infections. In two days an average of 200 small, brown spots appeared on the infected, non-treated flowers. In the same period, the infected, nystatin-treated flowers remained unspotted. In six to seven days, extensive mycelial growth appeared on the infected, nontreated orchids, and the conidia of *Botrytis* were readily visible when viewed under a dissecting microscope. On the other hand, the treated flowers developed, on the average, no more than two to three spots.

Nystatin was also effective in controlling the extension of already established fungal infections. Spotted orchids were cut from plants and sprayed with nystatin. After incubation at 4 C. at high humidity for 14 days, there was no increase in the size or number of spots on the treated flowers. In contrast, the untreated flowers showed extensive fungal spoilage.

The natural beauty of the orchid was not altered by application of nystatin. Spraying cut orchids daily with a suspension of nystatin at 300 units/ml. in 0.5 per cent dimethylsulfoxide for a period of one week left no visible residue on the flowers, and no signs of phytotoxicity were observed. Daily sprays of the suspension for 30 days on mature orchid plants with exposed root tips also gave no evidence of phytotoxicity.

To determine the possible utility of nystatin in other plant diseases, a group of phytopathogenic fungi was tested by the previously described agar dilution procedure. The data presented in table I indicate the broad antifungal activity displayed by nystatin.

In vitro data alone, however, are not completely indicative of the results obtained when nystatin is applied to the disease condition in or on the plant. Natti<sup>4</sup> has shown that nystatin is only moderately effective in inhibiting spore germination of the causative agent of downy mildew of broccoli, *Peronospora parasitica*, but in the actual greenhouse control of downy mildew, nystatin was one of the most effective in a group of 37 materials tested. Verona and Gambogi<sup>5</sup> showed that nystatin was effective in the control of *Pythium* infection of clover, although our in vitro data suggest it would be of little value in this disease.

Other investigators have utilized nystatin to control postharvest spoilage of fruits and vegetables. For example, DiMarco and Davis<sup>6,7</sup> found nystatin effective in the control of brown rot and *Rhizopus* rot of peaches and *Botrytis* and *Rhizopus* rots of strawberries. Fungal spoilage of dormant rose bushes in cold storage was prevented by the application of nystatin, according to Stessel;<sup>8</sup> and Gould<sup>9</sup> reported that it showed promise in controlling snow mold of turf. Smith<sup>10</sup> presented data showing nystatin effective in the protection of turf against *Corticium* disease (*Corticium fuciforme*). In view of its wide spectrum and lack of toxicity, further studies on the use of nystatin in the control of other plant diseases and spoilage conditions seem warranted.

#### SUMMARY

Nystatin was successfully used in the control of fungal spoilage of *Cattleya* orchids by direct application to the flower. A procedure was described for the experimental infection of orchids with spores of a *Botrytis* species. The high in vitro activity of

nystatin against phytopathogenic fungi suggests a broad application of this antifungal agent against plant diseases.

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#### BIBLIOGRAPHY

1. ANON.: Losses in Agriculture: A Preliminary Appraisal for Review, Agricultural Research Service, U.S.D.A., ARS-20-1, Washington, June, 1954.
2. DONOVICK, R.; PANSY, F. E.; STOUT, H. A.; STANDER, H.; WEINSTEIN, M. J., AND GOLD, W.: Some *in vitro* characteristics of nystatin (Mycostatin). In: STERNBERG, T. H., AND NEWCOMER, V. D., EDITORS: Therapy of Fungus Diseases, Boston, Little, Brown and Co., 1955, pp. 176-185.
3. FEDER, W. A.: Transit rot of vanda orchid blossoms, *Phytopathology* 42:373-376, 1952.
4. NATTI, J. J.: Control of downy mildew of broccoli with antibiotics and fungicides, *Plant Dis. Reporter* 41:780-788, 1957.
5. VERONA, O., AND GAMBOGI, P.: Intorno all'azione antimicotica della nistatina I. Azione su *Aspergilli*, *Penicilli* e funghi fitopatogeni, *Ann. sper. agrar.*, n.s. 11:193-209, 1957.
6. DiMARCO, G. R., AND DAVIS, B. H.: Prevention of decay of peaches with post-harvest treatments, *Plant Dis. Reporter* 41:284-288, 1957.
7. DiMARCO, G. R., AND DAVIS, B. H.: Prevention of decay of strawberries with post-harvest treatments, *Plant Dis. Reporter* 41:460-464, 1957.
8. STESSEL, G. J.: Botrytis control in stored rose stocks, *Plant Dis. Reporter* 42:396-398, 1958.
9. GOULD, C. J.: Turf diseases in western Washington, *Golf Course Reporter* 25:18-20, 1957.
10. SMITH, J. D.: Corticium disease fungicide trials, 1957, *J. Sports Turf Research Inst.* 9:367-368, 1957.

# An Evaluation of Nystatin and Candicidin Against a Standardized Systemic *Candida albicans* Infection in Mice

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Nystatin, a tetraene, and candicidin, a heptaene, are antifungal antibiotics produced by cultures of soil actinomycetes, *Streptomyces noursei* and *Streptomyces griseus*, respectively.<sup>1-3</sup>

The purpose of this paper is twofold: (1) to report the results obtained from an investigation of the effect of nystatin against a systemic *Candida albicans* infection in mice and (2) to present comparative evaluations of candicidin and nystatin against this infection.

## EXPERIMENTAL MATERIALS AND METHODS

**Mice.** The unit test group of mice consisted of 10 Carworth Farms, strain CFI, females, 6 to 8 weeks old, weighing 180 to 220 Gm./group. Mice were maintained on Purina laboratory chow.

**Infection.** *C. albicans*, strain 300, was used throughout these experiments. Infections were produced by intravenous injection of 0.2 ml. volume of  $10^{-1}$  suspensions of *C. albicans* in Sabouraud's liquid medium. Infecting doses contained  $3.0 \pm 0.5$  times  $10^6$  viable *Candida*, determined by plate counts. The mortality for untreated control mice was 99.9 per cent (1199/1200) within the span of one week post-infection. Table I summarizes daily mortality for all infected control mice used in these studies.

**Treatment.** INJECTION METHOD. Drugs used for treatment by injection were prepared by triturating in 0.2 per cent aqueous agar,  $pH \pm 4.5$ . Doses of 0.5 ml. volume were injected subcutaneously or intraperitoneally or were administered orally by gavage. Treatments were administered at 1, 4, 24, 28, 48, and 52 hours after infection, depending on the assigned schedule.

Nystatin suspensions, final  $pH$  4.0 to 5.5, were used at levels covering a 16- to 128-fold dosage range, graded twofold.

Candicidin, final  $pH$  4.0 to 8.0, was soluble at levels used, covering a 16- to 32-fold range, in twofold graded doses.

Mice treated by injection and that did not survive the prescribed treatment period were excluded from the calculations of mortality ratios.

**DRUG DIET METHOD.** Nystatin was tested by drug diet administration on schedules varying from 2 to 10 days. The drug was uniformly mixed in powdered Purina laboratory chow. The drug diets were dispensed in food hoppers, placed one to a cage, and were available ad libitum throughout the designated schedule of diet therapy. Nystatin in the diet was tested at twofold levels covering a 16- to 32-fold range.

The amount of drug intake was calculated as mg./Kg. of body weight per day.

Effectiveness of the drug was based on the survival ratios at 21 days, regardless of whether the mice lived through the entire period of therapy.

**Routine Test Procedure.** Over a period of several months, one or two evaluations were performed each week. Each test had from two to six groups of infected, untreated controls and one group of uninfected, untreated controls. Appropriate drug

TABLE I

*The Effect of Intravenous Infection with C. albicans 300, on Untreated Control Mice*

	Survival time in days postinfection							Total (20 days)
	1	2	3	4	5	6	7-20	
No. mice	885	139	73	59	31	8	4	1199/1200
% dead	73.7	11.6	6.1	4.9	2.6	1.0		99.9
Cumulative % dead		85.3	91.4	96.3	98.9	99.9		

controls were included in several tests, in which the mice were uninfected, but treated by the same method as the infected groups.

Duration of the test was 21 days. Mortality was recorded daily.

Several levels of the drugs were tested in replicate two or more times. Drug effectiveness was based on combined results of replicate tests at each level.

*Criteria of Drug Effectiveness.* A criterion of effectiveness of nystatin, the reference drug, was arbitrarily established as the survival of treated, infected mice for 21 days. Observation of tests was thus extended for two weeks beyond the death of all infected control mice.

TABLE II

*Percentage of Nystatin-Treated Mice That  
Harbored Viable Candida 22 Days Postinfection  
(Pooled Results of Two Tests)*

Nystatin, mg./Kg./dose	Subcutaneous dosage schedule	No. positive kidney cultures/ no. mice surviving 22 days*	Percentage positive CA300
512	1 dose	3/13	23
256	+1 hr.	7/10	70
128		3/4	75
64-4		All mice died before 22 days	—
256	2 doses	4/10	40
128	+1, +4 hr.	10/17	59
64		6/7	86
32		2/2	100
16-4		All mice died before 22 days	—
128	3 doses	2/10	20
64	+1, +4, +24 hr.	13/16	81
32		4/5	80
16		1/1	100
8-4		All mice died before 22 days	—
128	4 doses	1/8	13
64	+1, +4, +24, +28 hr.	9/15	60
32		9/10	90
16-4		All mice died before 22 days	—
128	5 doses	5/9	56
64	+1, +4, +24, +28, +48 hr.	7/14	50
32		11/13	85
16		3/5	60
8-4		All mice died before 22 days	—
128	6 doses	0/8	0
64	+1, +4, +24, +28, +48, +52 hr.	2/9	22
32		6/11	55
16		2/2	100
8-4		All mice died before 22 days	—
10 <sup>-1</sup> controls		120 mice died 1.2 to 1.9 days	—
Normal controls		0/20	0

\* All mice surviving the 21 day test period displayed symptoms of central nervous system disorders at time of sacrifice (22 days) when the kidney cultures were made.

Another criterion resulted from the observation of development of symptoms of central nervous system disorder during the second week postinfection in all mice treated with nystatin on all schedules by each route. Absence of these symptoms could readily be detected. Tests were also made to determine the percentage of mice that harbored viable *Candida* in the kidneys after subcutaneous administration of nystatin on the one- through six-dose schedules. In table II the results of two such tests are summarized, by drug levels and treatment schedules. There was a direct relationship between the size of the dose and the percentage of negative kidney cultures.

TABLE III

*Comparisons of the Effectiveness of Nystatin by Various Subcutaneous Dosage Schedules Against C. albicans 300 Intravenous Infection in Mice (Combined Results of 4 Tests); Survival on Twenty-first Day Postinfection*

Nystatin,* mg./Kg./dose	Cumulative, mg./Kg.	Subcutaneous dose schedule	Alive/total†	% effect
512	512	+1 hr.	27/40	67.5
256	256	(1 dose)	23/40	57.5
128	128		8/40	20.0
64	64		1/40	2.5
32	32		0/40	0
16	16		0/40	0
8	8		0/40	0
4	4		0/10	0
256	512	+1 and +4 hr.	29/30	96.7
128	256	(2 doses)	33/40	82.5
64	128		13/40	32.5
32	64		3/40	7.5
16	32		0/40	0
8	16		1/40	2.5
4	8		0/10	0
128	384	+1, +4 and	25/28	89.3
64	192	+24 hr.	30/40	75.0
32	96	(3 doses)	10/40	25.0
16	48		1/40	2.5
8	24		0/38	0
4	12		0/9	0
128	512	+1, +4, +24,	23/30	76.7
64	256	and +28 hr.	28/40	70.0
32	128	(4 doses)	20/40	50.0
16	64		5/40	12.5
8	32		1/37	2.7
4	16		0/8	0
128	640	+1, +4, +24	22/29	75.8
64	320	+28 and +48	24/39	61.6
32	160	hr.	18/40	45.0
16	80	(5 doses)	10/39	25.7
8	40		2/31	6.5
4	20		0/4	0
128	768	+1, +4, +24	20/30	66.7
64	384	+28, +48 and	23/40	57.5
32	192	+52 hr.	20/40	50.0
16	96	(6 doses)	5/35	14.3
8	48		0/23	0
4	24		0/3	0

Infected controls: 100 per cent (240/240) infected, untreated control mice were dead in an average of from 1.1 to 2.7 days/group of 10 mice.

Normal controls: 97.5 per cent (39/40) uninfected, untreated, "age-condition" control mice were alive on the twenty-first day when test was terminated.

\* Squibb's lot no. 7J69226.

† Total number of mice that received full prescribed treatment.

TABLE IV

Comparisons of the Therapeutic Effectiveness of Nystatin by Multiple Intraperitoneal Dosage Schedules Against the Systemic *C. albicans* Intravenous Infection in Mice (Combined Results of 4 Tests); Survival on Twenty-first Day Postinfection

Nystatin,* mg./Kg./dose	Cumulative mg./Kg.	Intraperitoneal dosage schedule	Alive/total†	% effect
32	32	+1 hr.	16/40	40.0
16	16	(1 dose)	1/40	2.5
8	8		0/40	0
4	4		0/40	0
32	64	+1, +4 hr.	29/40	72.5
16	32	(2 doses)	10/40	25.0
8	16		0/40	0
4	8		0/40	0
32	96	+1, +4, +24	37/40	92.5
16	48	hr.	18/40	45.0
8	24	(3 doses)	0/40	0
4	12		0/38	0

Infected controls: 100 per cent (80/80) infected, untreated control mice were dead in averages of from 1.2 to 2.0 days/group of 10 mice.

Normal controls: 100 per cent (30/30) uninfected, untreated, "age-condition" control mice were alive on the twenty-first day when test terminated.

\* Squibb's lot no. 7J69226.

† Total number of mice that received full prescribed treatment.

*Evaluation of Therapeutic Effect.* The results of replicate tests were combined and the median effective dose ( $ED_{50}$ ) was calculated by the method of Litchfield and Wilcoxon.<sup>4</sup> The activity of each drug, expressed as mg./Kg./dose, was determined for each method of drug administration on all schedules.

The relative potency of candicidin was determined, where possible, by dividing the  $ED_{50}$  of candicidin into the corresponding value of nystatin.

## RESULTS

The effect of subcutaneous administration of nystatin on one- through six-dose schedules against *C. albicans* is shown in tables III and VII. Nystatin at 512 mg./Kg. (maximum tolerated single subcutaneous dose) was less effective when given as one dose than when divided into two or four doses. At the 128 mg./Kg./dose level,

TABLE V

The Effect of Nystatin Administered in the Diet on a Single Schedule Against the Systemic *C. albicans* Infection in Mice

% nystatin in diet	Drug intake, mg./Kg./day	Alive/total*	% effect
Drug diet schedule: —4 to +6 Days†			
0.8	1300	5/10	50
0.4	690	4/10	40
0.2	370	1/10	10
0.1	170	0/10	0
0.05	90	1/10	10
0.8 controls	1300	10/10	Uninfected
10 <sup>-1</sup> controls	No drug	0/20	—
Normals	No drug	10/10	Uninfected

\* Survival as of the twenty-first day postinfection. Nystatin batch C6167.

† Four days before to six days after infection.

nystatin was progressively more effective through three doses, but decreasingly effective from four to six doses. Increasing the number of doses beyond three did not decrease the ED<sub>50</sub>, but produced more tissue damage and general debilitation in uninfected as well as infected mice. For this reason, the three-dose schedule was selected to be used routinely.

Comparative effectiveness of nystatin injected intraperitoneally on the one-, two-, and three-dose schedules against systemic *C. albicans* is shown in tables IV and VII. One dose at maximum tolerated (M.T.D.) of 32 mg./Kg. was less than 50 per cent effective. Two doses of 32 mg./Kg./dose increased the effect twofold. Three doses at

TABLE VI

*The Effect of Nystatin and Candicidin Administered by Various Dosage Schedules and Routes on the Survival of Mice with a Systemic C. albicans Infection*

Drug	Schedule and route	Survival on the 21st day postinfection			
		mg./Kg./dose	Alive/total*	% effect	Mean survival†
Nystatin	3 doses subcutaneously (1, 4, and 24 hr. after infection)	256	32/39	82.0	3.8
		128	33/40	82.5	10.4
		64	17/40	42.5	7.0
		32	14/40	35.0	6.7
Candicidin		64	1/20	5.0 (toxic)	5.9
		32	25/40	62.5	6.1
		16	19/40	47.5	6.6
		8	15/39	38.5	12.2
		4	4/19	21.0	9.0
Nystatin	1 dose, subcutaneously (1 hr. after infection)	512	12/20	60.0	7.7
		256	11/20	55.0	8.8
		128	11/20	55.0	8.5
		64	3/10	30.0	6.0
Candicidin		512	12/20	60.0 (toxic)	8.1
		256	30/30	100.0	—
		128	26/30	86.7	11.3
		64	23/30	76.7	10.1
		32	4/9	43.4	11.8
		16	3/10	30.0	13.1
Nystatin	3 doses, intraperitoneally (1, 4, and 24 hr. after infection)	16	27/38	71.0	4.4
		8	18/40	45.0	8.5
		4	5/40	12.5	9.4
		2	2/40	5.0	6.9
		2.0	0/5	0 (toxic)	2.0
Candicidin		1.0	14/40	35	14.2
		0.5	0/39	0	6.7
		0.25	0/39	0	4.9
		0.125	0/25	0	2.1
Nystatin	3 doses, orally (1, 4, and 24 hr. after infection)	1024	0/9	0	3.2
		512	0/6	0	3.0
Candicidin		256	1/6	17	5.4
		128	0/5	0	3.4
		64	0/2	0	2.5
		32	0/3	0	1.7

Infected controls: 100 per cent (160/160) untreated, infected (with 10<sup>-1</sup>) control mice were dead with mean survival times from 1.1 to 3.0 days (per group of 10) postinfection; mean, 1.9;† 87.5 per cent (35/40) untreated, infected (with 10<sup>-2</sup>) control mice were dead with mean survival times from 6.9 to 14.9 days (per group of 10) postinfection; mean, 11.0.†

"Age-condition" controls: 100 per cent (40/40) untreated, uninfected mice were alive at 21 days when test was terminated.

\* Number of mice surviving 21 days/number of mice that received full prescribed treatment.

† Averages of the mean survival times of mice that died (in replicate tests) in days post-infection.

TABLE VII

*Dosage-Effect Curve Parameters of the Standard Nystatin, Against Systemic C. albicans*

Dosage schedule	ED <sub>50</sub> , mg./Kg./dose*	Slope function†
Multiple subcutaneous dosage schedules		
Batch 7J69226		
1	230 (190–280)	1.87 (1.57–2.22)
2	80 ( 66– 96)	1.83 (1.60–2.09)
3	53 ( 43– 65)	1.91 (1.65–2.22)
4	42 ( 32– 55)	2.46 (1.94–3.13)
5	45 ( 33– 60)	3.56 (2.52–5.02)
6	51 ( 39– 67)	2.74 (2.12–3.54)
Multiple intraperitoneal dosage schedule		
Batch 7J69226		
1	41 (estimated)	—
2	23 ( 19– 27)	1.77 (1.53–2.06)
3	17 ( 14– 21)	1.58 (1.39–1.80)
Three intraperitoneal dosage schedule		
Batch C6167		
3	10 ( 7– 12)	2.33 (1.69–3.22)

\* Median effective dosage.

† Slope function =  $\frac{ED_{94}/ED_{50} + ED_{50}/ED_{16}}{2}$ .

M.T.D. gave good protection with one lot of nystatin (7J69226), but were lethal for infected mice with another lot (C6167). Both lots were toxic for a small percentage of uninfected mice. The limited safety margin for intraperitoneal administration of nystatin in this system made this method undesirable for routine use.

Nystatin was completely ineffective by gavage in three doses of 1024 mg./Kg./day (M.T.D.). In the drug diet, at nontoxic percentages, as indicated by weight loss and appearance of the mice, nystatin was therapeutically inactive. Levels ranging from 0.05 to 1.6 per cent on schedules including 2 to 10 days of therapy were tested. Above 0.4 per cent in the diet, nystatin was not well tolerated. Results of a typical test are given in table V. The mice on 0.8 and 0.4 per cent of nystatin in the diet were extremely emaciated, and intravenous infection was difficult, if possible at all.

Candididin was compared with nystatin by subcutaneous, intraperitoneal, and oral administration. The data in tables VI and VIII indicate that candididin was more toxic and more effective than nystatin. One subcutaneous dose of candididin at 256 mg./Kg. protected 100 per cent of the mice, while nystatin at twice the

TABLE VIII

*Dosage-Effect Curve Parameters of Candididin and Nystatin Compared by Parenteral Routes on Various Dosage Schedules*

Drug	Median effective dose, mg./Kg./dose	Slope function	Relative activity
Three subcutaneous dosage schedule			
Nystatin	65 (48–88)	3.3 (2.0– 5.2)	1.0
Candididin	17 (11–25)	5.4 (2.7–10.9)	3.8 (2.3–6.3)
Single subcutaneous dose			
Nystatin	>100	—	—
Candididin	34 (22–51)	2.9 (1.8– 4.7)	—
Three intraperitoneal dosage schedule			
Nystatin	10 ( 7–12)	2.3 (1.7– 3.2)	—
Candididin	> 1	—	—

dosage protected only 60 per cent of the mice. On the three-dose schedule by the subcutaneous route, candicidin was eight times as toxic as nystatin. On a relative dosage basis, candicidin effectiveness was approximately four times that of nystatin with three subcutaneous doses. Except at the maximum tolerated dosage (1.0 mg./Kg./dose) on the three-dose schedule of intraperitoneal administration, candicidin did not save any mice, but increased the survival time over that of nystatin.

Oral administration of each drug was ineffective at maximum tolerated dosages given by gavage (table VI).

#### SUMMARY

A systemic *C. albicans* infection, regularly lethal for untreated control mice within one week postinfection, was standardized. Nystatin was investigated to determine its optimum effect against this infection.

Several routes and dosage schedules of administering nystatin to mice against this infection were tested. A three-dose schedule subcutaneously was adopted because of greater tolerance and better physical condition of the test mice. Fewer than three doses were less effective, and more than three doses produced additive tissue damage and general debility in mice. Effects of intraperitoneal dosage schedules were less reproducible, and oral administration was ineffective.

The effectiveness of candicidin was compared by the various routes, using nystatin as the reference standard. Although more toxic for mice, candicidin was more effective than nystatin when administered in three subcutaneous doses. The dose response was greater with candicidin than with nystatin on the single subcutaneous dosage basis. Candicidin and nystatin effects were equally nonreproducible by intraperitoneal injection. Oral dosage of each was ineffective.

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#### BIBLIOGRAPHY

1. HAZEN, E. L., AND BROWN, R.: Two antifungal agents produced by a soil actinomycete, *Science* 112:423, 1950.
2. HAZEN, E. L., AND BROWN, R.: Fungicidin, an antibiotic produced by soil actinomycete, *Proc. Soc. Exper. Biol. & Med.* 76:93-97, 1951.
3. LECHEVALIER, H.; ACKER, R. F.; CORKE, C. T.; HAENSELER, C. M., AND WAKSMAN, S. A.: Candicidin, a new antifungal antibiotic, *Mycologia* 45:155-171, 1953.
4. LITCHFIELD, J. T., JR., AND WILCOXON, F.: A simplified method of evaluating dose-effect experiments, *J. Pharmacol. & Exper. Therap.* 96:99-113, 1949.

# Comparative Effectiveness of Antibiotic Dusting Powders in Candidal and Non-Candidal Diaper Rashes

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The important role played by *Candida albicans* in the etiology of diaper rashes is becoming increasingly well recognized.<sup>1-4</sup> In practice, the clinical picture of cutaneous candidiasis is often obscured by ammoniacal dermatitis, by skin reaction to over-treatment or by other secondary pathology. In such cases, the specific diagnosis cannot be obtained except by demonstration of *C. albicans* in scrapings and cultures from the skin lesions.

This type of laboratory investigation is rarely available to the pediatrician in his treatment of the patient seen at home or in the office. What procedure should be followed, then, in the therapeutic management of the child with a diaper rash which is suspected due to *C. albicans* without laboratory confirmation of the diagnosis, or of the infant with a dermatosis of obscure and possibly multiple origin? Now that we know how often this yeast causes diaper rash, and how to treat it by means of the newer antifungal antibiotics, it would be well to know what would be the effect of treating both candidal and non-candidal diaper dermatoses with specific antifungal medication.

Pediatricians would welcome preparations that may be given safely and effectively in a variety of diaper rashes of obscure etiology, which cannot be treated immediately with specific remedies. Such medication should combine specific antimycotic action with antibacterial agents designed to relieve secondary infections, and neither agent should interfere with the action of the other. This type of medication could be expected to be effective in both candidal and a variety of non-candidal diaper dermatoses and harmful in neither type.

Since dusting powder is valuable in the treatment of moist skin lesions, it seemed of practical interest to compare medicated and non-medicated powders in the management of persistent candidal and non-candidal diaper rashes.

## METHOD

The most reliable way of evaluating new medications is the blind study, in which the physician tests a series of preparations, including a control, whose exact composition is made known to him only after the study is completed.

In the present study three types of dusting powders were used to treat candidal and non-candidal diaper dermatoses. The powders were designated as "A," "E" and "C," respectively. After the study was completed, the writers were informed that powder A contained 3 per cent nystatin-neomycin-gramicidin; powder E contained 100,000 units of nystatin per Gm.; and powder C was talcum powder, which could be considered to represent the unmedicated control preparation.

Seventy children, ranging in age from 10 days to 2½ years, were treated. All had been treated unsuccessfully with various home remedies. Cultures were taken from the lesions in all cases prior to initiation of therapy.

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*C. albicans* was isolated from the skin lesions of 28 patients. These cases were classified as cutaneous candidiasis on the basis of clinical appearance of the lesions and of cultural findings. The average duration of the candidal lesions before therapy was begun was 12.2 days. Oral thrush was, or had been, present in 9 of the 28 patients in this group.

No fungus was isolated from the lesions in 42 cases, although 2 patients in this group had a history of previous cutaneous candidiasis, and oral thrush was, or had been, present in 5 children. The skin conditions identified included seborrheic, contact, ammoniacal dermatitis, and generalized eczema, with an average duration of 33 days.

In order to obtain uniform conditions of therapy, and to rule out irritation due to faulty techniques, the mothers were instructed to employ a diaper service instead of home laundry and to refrain from using rubber or plastic panties on the patients during the period of treatment.

The test period was set at 15 days, and the patients were seen at five day intervals during that time. The powder was applied three times daily. Each test group was treated with one of the powders, and comprised cases of both cutaneous candidiasis and non-candidal diaper rashes.

When no response was evident, or the condition appeared aggravated after five days' therapy, the medication was discontinued, and the result classed as a failure, with the arbitrary numerical value of 0. This time interval was judged sufficient on the basis of our previous experience with antimycotic ointments.<sup>5</sup>

Noticeable regression of the lesions after 15 days was classed as "improvement," with the numerical value 1. Almost complete or complete regression was designated as "marked improvement," with the numerical value 2.

RESULTS AND CONCLUSIONS

The therapeutic results obtained with the three dusting powders under clinical trial are tabulated in table I for the candidal and in table II for the non-candidal dermatoses.

On inspection of these figures, it can be seen that, while the numbers of cases are small, in both candidal and non-candidal dermatoses the percentage of cases showing marked improvement with the two medicated powders is more than twice that of the talcum powder controls, and that the failure rate is less with the two antifungal antibiotic powders tested. This difference is particularly marked in the case of

TABLE I  
*Effectiveness of 3 Dusting Powders in Candidal Diaper Rash*

Powder	Composition	Total cases	Marked improvement		Improved		Failure	
			No.	Per cent	No.	Per cent	No.	Per cent
A	3% nystatin-gramicidin-neomycin	17	8	47	9	53	0	0
E	100,000 units nystatin per Gm.	5	2	40	3	60	0	0
C	Unmedicated talcum powder: control	6	1	17	0	0	5	83

TABLE II  
*Effectiveness of 3 Dusting Powders in Non-candidal Diaper Rash*

Powder	Composition	Total cases	Marked improvement		Improved		Failure	
			No.	Per cent	No.	Per cent	No.	Per cent
A	3% nystatin-gramicidin-neomycin	24	4	17	17	70	3	13
E	100,000 units nystatin per Gm.	6	1	17	4	66	1	17
C	Unmedicated talcum powder: control	12	1	8	7	58	4	34

candidal diaper rash, where there were no failures with both the medicated preparations, while talcum powder treatment lead to a failure rate of 83 per cent.

In order to give some weight to all cases in this series, effectiveness may be expressed numerically in terms of arbitrary values, such as for example "2" for marked improvement, "1" for improved and "0" for failure (the actual numbers chosen, if linear, are immaterial). In the case of candidal diaper rash, we find in this manner that the average effectiveness of preparation "A" was 1.5 and of preparation "E," 1.4, just about halfway between improved and markedly improved in both cases. For the talcum control, on the other hand, the corresponding value is 0.3, indicating an average effectiveness very close to failure. The preliminary conclusion may be drawn therefore that in this series of cases the incorporation of nystatin into a dusting powder, whether with or without the addition of gramicidin and neomycin, markedly enhanced the therapeutic effect.

In the case of the non-candidal dermatoses, the number of markedly improved cases is proportionately twice as large in the case of the medicated as of the non-medicated dusting powders. When the same numerical measure of effectiveness is used, however, the average effectiveness for both medicated powders is exactly 1.0 (improved), and only a little less, 0.8, for the talcum powder controls. The evidence at hand is therefore insufficient to suggest that nystatin or gramicidin-neomycin may be of therapeutic value in these cases. It can be stated with assurance, on the other hand, that neither nystatin nor nystatin-gramicidin-neomycin dusting powders exert a deleterious effect upon the course of non-candidal diaper rash.

There were no side reactions noted in any of the patients treated in this series. In particular, there occurred no aggravation of the skin condition attributable to any of the three dusting powders in any of the 70 patients under treatment.

#### SUMMARY

Three types of dusting powders containing, respectively, nystatin, nystatin-gramicidin-neomycin, and talcum powder without specific medication, were used to treat 70 cases of diaper rash for 5 to 15 days. By laboratory examination, 28 of these were identified as candidal, and 42 as non-candidal in etiology. Both types of diaper rash were subjected to similar therapeutic management, mainly in an effort to determine whether the use of specific antifungal therapy is helpful or harmful in non-fungal diaper rash.

Cutaneous candidiasis responded well to the medicated, but not to the nonmedi-

cated preparation. Non-candidal diaper rash showed some improvement with all three types of dusting powder. These findings suggest that medicated dusting powders containing nystatin may be used to advantage not only in cases of diaper rash of proved candidal etiology, but also in diaper rashes of other, mixed or undetermined origin, especially when the presence of *C. albicans* is suspected but cannot be confirmed.

#### BIBLIOGRAPHY

1. DOBIAS, B.: Cutaneous moniliasis in pediatrics; diagnosis and treatment with nystatin. *In: Therapy of Fungus Infections, An International Symposium*, Boston, Little, Brown and Co., 1955; p. 205.
2. BOUND, J. B.: Thrush napkin rashes, *Brit. M. J.* 1:782, 1956.
3. ROBINSON, R. C.: Cutaneous moniliasis in infants, *J. Pediat.* 50:721, 1957.
4. KOZINN, P. J.; TASCHDJIAN, C. L.; DRAGUTSKY, D., AND MINSKY, A.: Cutaneous candidiasis in early infancy and childhood, *Pediatrics* 20:827, 1957.
5. KOZINN, P. J.; TASCHDJIAN, C. L.; DRAGUTSKY, D., AND MINSKY, A.: Treatment of cutaneous candidiasis in infancy and childhood with nystatin and amphotericin B. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, pp. 128-134.

# Antibiotics Against Plant Disease. VI. An Agar-Diffusion Method for Determining the Effects of Chemicals on Germination of Bean-Rust Uredospores

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Within the past few years, much interest has been generated in the use of antibiotics in areas other than those concerned with human disease therapy. Research in these new areas already has led to the use of antibiotics for animal feed supplementation, for food preservation, and for control of certain plant diseases. Each of these developments has been accompanied by many new and interesting problems. For example, in a plant disease antibiotics-screening program, one is faced with the problem of how best to handle obligate parasites, such as the rusts and mildews. Is it possible to screen in the laboratory for materials effective against these forms, and if so, what meaning would the results have? Will materials found effective by laboratory methods be effective in greenhouse or field trials? Having had little success with the conventional spore germination tests used with these organisms, we have taken a somewhat different approach in attempting to find answers to these questions. The method outlined herein may offer new leads to other investigators engaged in antibiotic screening. It also may suggest a new lead to investigators attempting to develop methods for cultivation of certain obligate parasites on laboratory media.

In research directed toward the isolation and purification of the principle or principles responsible for the marked anti-bean-rust activity of the antibiotic F-17 mixture, <sup>1-4</sup> experiments were made to develop a laboratory assay method to follow the progress of the work. Initial attempts to apply the conventional plate germination count method<sup>5</sup> were unsuccessful, although part of the problem was solved by using substrata other than the recommended tap water agar. Under certain conditions, germinating uredospores of the bean-rust organism (*Uromyces phaseoli typica* Arthur) formed an abundance of long, intertwined aerial germ tubes simulating the aerial growth of saprophytic molds growing on laboratory media. The gross appearance of these forms suggested the possibility of developing an agar diffusion assay method. Although not entirely satisfactory for the purpose originally intended, the method does offer promise for use in selecting chemicals with antirust activity, particularly those that tend to suppress germination of rust uredospores. Our results also suggest that it might be useful in studies leading toward the culture of obligate parasites on laboratory media.

## MATERIALS AND METHODS

*Organism.* Most of the work reported here was conducted with fresh (not more than two weeks old), relatively dry uredospores of the bean-rust organism, *U. phaseoli typica* Arthur. In several experiments, uredospores of wheat stem rust, *Puccinia graminis* Pers. (Race 15 B), and several races of oat crown rust, *Puccinia coronata* Corda, were used. Uredospore collections were stored in a refrigerator at 3 to 5 C. from receipt until use.

*Medium (V-8 Juice-Tap Water Agar).* V-8 juice, 100 ml., Difco Noble agar,

20.0 Gm., tap water, 900 ml., were used. The pH was not adjusted. The mixture was steamed at 100 C. for 15 minutes, dispensed, and allowed to solidify.

The steam treatment became a routine procedure after it was found that autoclaved media apparently contained an inhibitor that prevented subsequent germination of the uredospores. Usually, 10 ml. of medium was dispensed into each 100 by 15 mm. Petri dish and allowed to solidify with the dish cover removed. For detection of activity on developed paper chromatogram strips, large rectangular trays of the usual type were employed.

Difficulties encountered in using the conventional tap water agar for germination of rust uredospores led to a survey of a number of substrata in order to select one most suitable for obtaining consistent and generally high levels of germination. Of a number of media studied, the V-8 juice-tap water agar proved best.

*Inoculation.* Initially, a suspension of uredospores was dispensed into various liquids, with or without various surface-active agents, but the uredospores tended to clump rapidly, and the resultant clusters of spores prevented accurate germination counts. Accordingly, the use of liquid inocula was abandoned in favor of dispersal of relatively dry uredospores as outlined later. The method for dispersal was first used by H. G. Tanner at Fort Detrick, Frederick, Maryland, for a somewhat similar purpose.

Spores were dispensed into no. 5 gelatin capsules with a spatula tamper and fired from a carbon dioxide target pistol (Crossman Model 116, 0.22 caliber Target Pistol, Crossman Arms Company, Inc., Rochester, New York) into a confined area over exposed plates. The pistol was modified with a cross-wire at the muzzle. Upon striking the cross-wire, the capsule ruptured, allowing a uniform "fall-out" of the spores. The number of capsules shot into a box depended on the size of the box and the number of Petri dishes at the bottom. Approximately 50 mg.

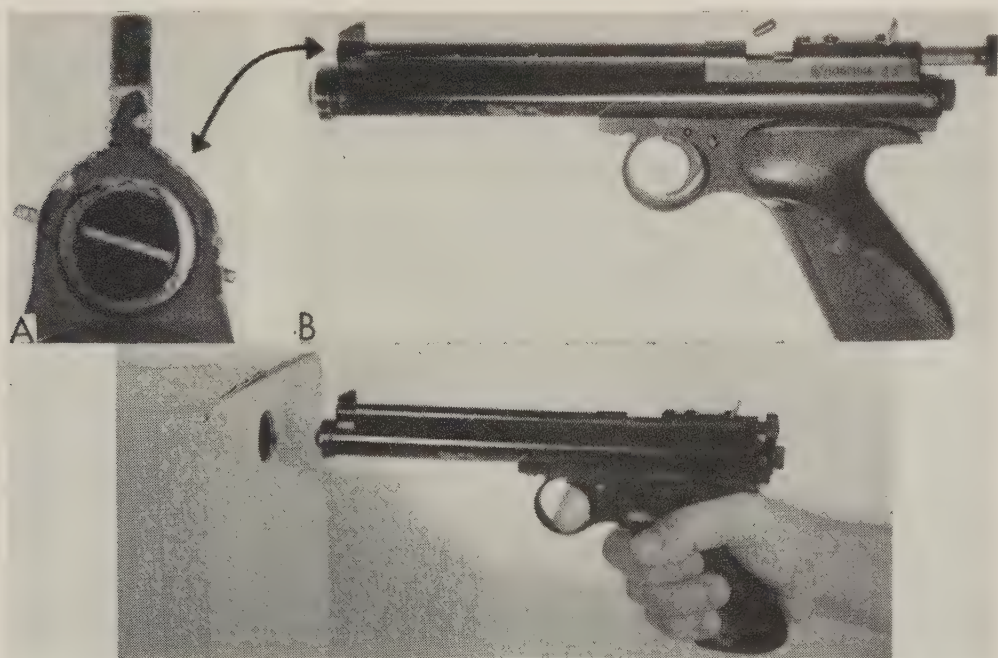


FIG. 1. Illustrated are the materials necessary for carrying out rust uredospore agar diffusion assays: A, front view of muzzle of pistol showing position of cross-wire for rupturing capsule; B, carbon dioxide pistol with loaded capsule, cross-wire is placed near end of muzzle; C, box into which capsule is fired.

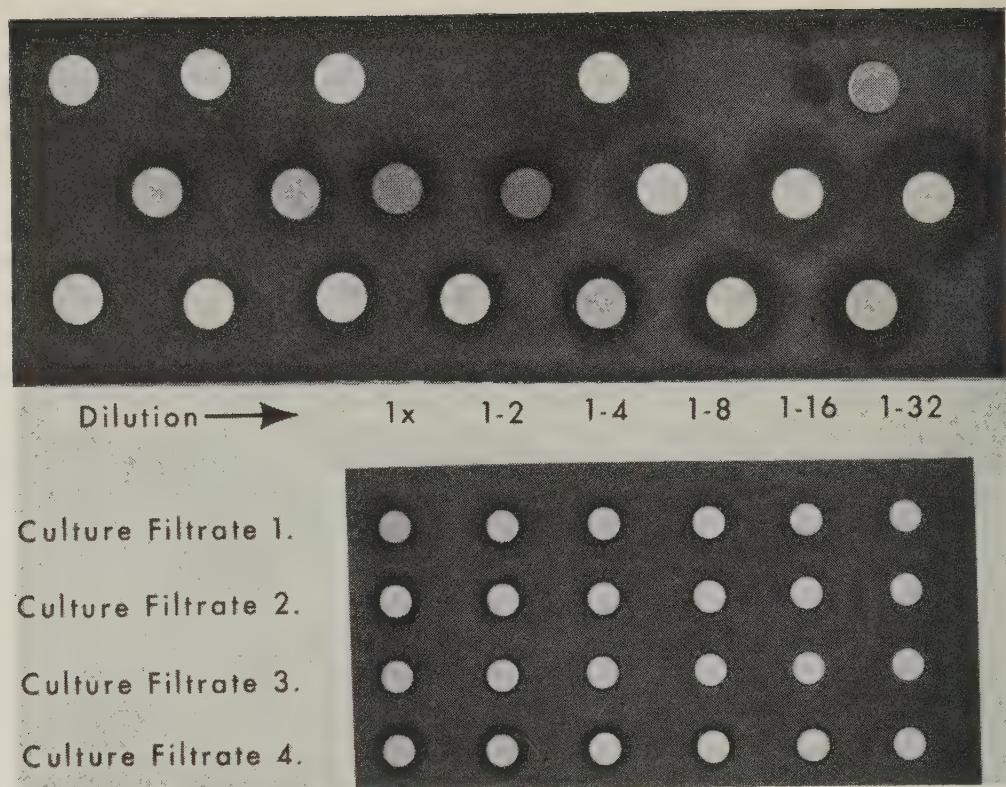


FIG. 2. Shown are representative results obtained on bean-rust germination assay of various antibiotic-containing culture filtrates: Top, 12.7 mm. paper discs saturated with culture filtrate; Bottom, 6.35 mm. paper discs saturated with various dilutions of four culture filtrates from top to bottom, 1X through 1-32 from left to right.

of spores loaded into one capsule was sufficient for a box 7 by 7 by 15 inches containing two 100 by 15 mm. Petri dishes. For larger boxes with a greater number of dishes, more than one 50 mg. capsule was necessary. Preliminary experiments were run to determine the optimal number of capsules to shoot into a particular box. Materials required in the method are illustrated in figure 1.

After each capsule was fired, an exposure time of one minute allowed the spores sufficient time to fall onto the surface of the medium. After the uredospores had settled, the plates were removed, covered, and incubated in the dark at  $20 \pm 1$  C. for 16 to 18 hours. To maintain the high relative humidity required for germination, the incubator was equipped with water pans. Occasional readings of a hygrometer installed in the incubator always indicated a relative humidity exceeding 85 per cent.

*Estimation of Potency of Test Agents.* Paper discs saturated with test materials, small glass cylinders containing test materials, or paper chromatogram strips may be placed on the surface of the medium prior to inoculation. After incubation, the diameters of zones of inhibition or stimulation can be measured or zones of activity on paper strips can be detected.

## RESULTS

Typical assay plates obtained using the procedures outlined are illustrated in figure 2. A typical bioautograph is illustrated in figure 3.

*Effect of Various Materials on Bean-Rust Germination.* In several experiments a number of known antibiotic-containing culture filtrates, purified antibiotics, agricultural chemical formulations, and other chemicals were assayed to determine their effect on bean-rust germination using the method described. Representative results obtained in these experiments are given in table I.

#### DISCUSSION

The assay method described offers promise for the screening of potential anti-rust antibiotics and other chemicals, and it should prove valuable in the isolation and purification of compounds that suppress uredospore germination. This method may also suggest new approaches for investigators working with certain obligate parasites in the laboratory. Obligate parasites other than bean rust can no doubt be exploited, because the results of preliminary experiments indicate the possible utility of the method with wheat stem rust (Race 15 B) and several races of oat crown rust.

In view of the studies of Allen<sup>6,7</sup> and French et al<sup>8,9</sup> concerning the role of certain chemicals that cause or prevent self-inhibition of uredospore germination, the techniques that we used undoubtedly can be improved. In some of our experiments, the addition of coumarin to the medium resulted in higher germination percentages and denser aerial mats. We are unable to determine whether the role of the V-8 juice medium is to supply suitable physical conditions or chemical factors required for germination. It may act by suppressing factors that cause self-inhibition. Most of the media containing tomato fractions as one ingredient served to increase germination of spores over that of controls, indicating the presence of some stimulatory factor in the tomato product. The observation that medium sterilized under pressure decreased spore germination is of particular interest. If the amount of V-8 juice was increased from the normal 10 to 20 per cent (v/v) prior to autoclaving, spore germination was improved. This would seem to indicate either a change in physical nature or heat lability of some component. The modified treatment of steaming the medium for 15 minutes at 100 C. did not result in serious contamination problems, since the period of incubation and low temperatures required did not favor the formation of macroscopically visible colonies of contaminants.

When one compares the results obtained with the plate germination counting test, the hanging drop germination counting test, greenhouse tests with infected bean plants, and the new method described, it appears that this assay is not as



FIG. 3. A bioautograph of antibiotic-containing culture filtrate using *Uromyces phaseoli typica* as test organism is illustrated.

TABLE I  
Effect of Test Materials on Bean-Rust Germination as  
Determined by Paper Disc, Agar-Diffusion Assay\*

Test material	Zone of Inhibition	
	Plate 1, mm.	Plate 2, mm.
Culture filtrates		
Antibiotic F-14 culture filtrate 1X	22.0	21.7
Antibiotic F-17 culture filtrate 1X	17.8 hazy	17.5 hazy
1-2 dilution	16.5 hazy	16.9 hazy
1-4 dilution	16.3 hazy	16.2 hazy
1-8 dilution	14.0 hazy	trace, hazy
1-16 dilution	0	0
Antibiotic F-21 culture filtrate 1X	19.1	19.3
Purified antibiotics†		
Candicidin, 1000 µg./ml.	20.0	
Endomycin, 200 µg./ml.	trace	
Thiolutin, 10 µg./ml.‡	20.0	
Acti-dione RZ§	42.0 irregular	
Acti-Spray, 1000 µg./ml.	0	
Acti-dione Ferrated§	0	
Other materials		
Mercuric chloride, 1:1000	20.0	
Phenol, 1%	29.0 very hazy	
Hydrogen peroxide (10 volumes)	35.5	
Polysorbate 80, 1%	0	
Gibberellin, 200 µg./ml.	0	

\* In some experiments, the V-8 juice agar was supplemented with coumarin (2 mg./ml.). This addition did not appear to affect assay results, but in some cases it stimulated germination of the uredospores.

† At a concentration of 200 µg./ml., the following antibiotics gave no inhibition: bacitracin, chloramphenicol, chlortetracycline, cinnamycin hydrochloride, cycloheximide, duramycin hydrochloride, neomycin, Netropsin, nystatin, oxytetracycline, penicillin G, polymyxin, streptomycin, and viomycin.

‡ Thiolutin was not soluble to the extent indicated. Paper discs were saturated with the supernatant solution obtained on adding the antibiotic to distilled water at the concentration indicated.

§ The agricultural and chemical formulations were prepared as directed on the labels.

sensitive as the others are. Antibiotic F-17 mixture culture filtrates markedly limit germination at dilutions as high as 1:480 in plate germination counting tests and in hanging drop germination counting tests. In plant tests, conducted by J. W. Mitchell, a similar degree of response is noticed (marked activity at dilutions of about 1:100). In our agar-diffusion assay, culture filtrates showed no activity at dilutions beyond 1:8. There is some evidence that the plant tests and the agar-diffusion assays are measuring different activities. Samples effective in plant tests are not necessarily effective in the agar-diffusion assay, and vice versa. Fractions obtained from the antibiotic F-17 culture filtrate, which have shown concentrated anti-bean-rust activity in plant tests, have been found actually to stimulate germination of uredospores in hanging drop assays.

#### SUMMARY

A method is described for determining the effects of chemicals on germination of bean-rust (*U. phaseoli typica* Arthur) uredospores. The medium consists of 10 per cent V-8 juice (v/v), 2 per cent Difco Noble agar, and tap water. Test

materials are placed on the surface of the solidified medium and inoculation is accomplished by firing relatively fresh uredospores, contained in a gelatin capsule, into a confined area by means of a 0.22 caliber carbon dioxide target pistol modified with a cross-wire at the muzzle. Inoculated, covered plates are incubated in the dark in a moist atmosphere for 16 to 18 hours at  $20 \pm 1$  C.

After incubation, the surface of the medium is covered with a white network of germ tubes simulating the aerial growth obtained with saprophytic fungi on solid substrata. Where effective chemicals have been applied directly with saturated paper discs or after development on paper chromatograms, zones of stimulation or inhibition are visible.

The principal difficulty encountered has been the inconsistent germination responses of the uredospores. The season and manner of collection and storage of spores or the self-inhibitory factors produced by the spores may be possible causes. Further studies are in progress.

#### ACKNOWLEDGMENT

We are greatly indebted to Dr. J. W. Mitchell, Head, Growth Regulator and Antibiotic Laboratory of the Crops Research Division, for supplying us with uredospores at biweekly intervals. The uredospores of wheat stem rust and several races of oat crown rust were kindly supplied by Drs. W. Q. Loegering, Field Crops Research Branch, U. S. Department of Agriculture, and M. D. Simons, Crown Rust Identification Laboratory, Iowa State College, respectively.

#### BIBLIOGRAPHY

1. PRIDHAM, T. G.; LINDENFELSER, L. A.; SHOTWELL, O. L.; STODOLA, F. H.; BENEDICT, R. G.; FOLEY, C.; JACKSON, R. W.; ZAUMEYER, W. J.; PRESTON, W. H., JR., AND MITCHELL, J. W.: Antibiotics against plant disease. I. Laboratory and greenhouse survey, *Phytopathology* 46:568-575, 1956.
2. PRIDHAM, T. G.; SHOTWELL, O. L.; STODOLA, F. H.; LINDENFELSER, L. A.; BENEDICT, R. G., AND JACKSON, R. W.: Antibiotics against plant disease. II. Effective agents produced by *Streptomyces cinnamomeus* forma *azacoluta* f. nov., *Phytopathology* 46:575-581, 1956.
3. SHOTWELL, O. L.; STODOLA, F. H.; MICHAEL, W. R.; LINDENFELSER, L. A.; DWORSCHACK, R. G., AND PRIDHAM, T. G.: Antibiotics against plant disease. III. Duramycin, a new antibiotic from *Streptomyces cinnamomeus* forma *azacoluta*, *J. Am. Chem. Soc.* 80:3912-3915, 1958.
4. LINDENFELSER, L. A.; PRIDHAM, T. G.; SHOTWELL, O. L., AND STODOLA, F. H.: Antibiotics against plant disease. IV. Activity of duramycin against selected microorganisms. In: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 241.
5. ALCORN, S. M., AND ARK, P. A.: The effect of candicidin on plant pathogenic fungi, *Plant Dis. Rep.* 38:705-709, 1954.
6. ALLEN, P. J.: The role of a self-inhibitor in the germination of rust uredospores, *Phytopathology* 45:259-266, 1955.
7. ALLEN, P. J.: Properties of a volatile fraction from uredospores of *Puccinia graminis* var. *tritici* affecting their germination and development. I. Biological activity, *Plant Physiol.* 32:385-389, 1957.
8. FRENCH, R. C.; MASSEY, L. M., JR., AND WEINTRAUB, R. L.: Properties of a volatile fraction from uredospores of *Puccinia graminis* var. *tritici* affecting their germination and development. II. Some physical and chemical properties, *Plant Physiol.* 32:389-393, 1957.
9. FRENCH, R. C., AND WEINTRAUB, R. L.: Pelargonaldehyde as an endogenous germination stimulator of wheat rust spores, *Arch. Biochem.* 72:235-237, 1957.

# The Development of Strains of *Candida albicans* and *Coccidioides immitis*, which are Resistant to Amphotericin B

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Amphotericin B, a polyene antifungal agent, is one of the few agents available that has activity in cases of systemic mycoses. Its future usefulness will depend upon the degree to which resistant strains of fungi are developed. This is of critical importance in the treatment of systemic mycoses as these are chronic conditions in which therapy is administered over a long period of time. Stout and Pagano<sup>4</sup> reported that they were able to develop a resistant strain of *Candida albicans* in only one of the five strains tested using nystatin, also a polyene antibiotic. Donovan and his co-workers were not successful in their attempts.<sup>1</sup> Littman et al<sup>2</sup> could not develop strains of *C. albicans* that were resistant to nystatin or amphotericin B, although they were able to accomplish this with other species of *Candida*, namely, *C. tropicalis*, *C. guilliermondii*, and *C. krusei*. They also reported that there were varying degrees of cross resistance between the nystatin and amphotericin B resistant strains and the other polyene antibiotics.

No report has been made of the development of resistance to amphotericin B or nystatin by other more pathogenic fungi, such as *Coccidioides immitis*, *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Cryptococcus neoformans*.

## MATERIALS AND METHODS

*Test Organisms.* Three strains of *C. albicans* were employed in this study; 178-1, 178-4, and 178-12 (Squibb 1539). McNall et al<sup>3</sup> tested 21 strains and found these to be the most sensitive to amphotericin B. The strains of *C. immitis* used were 200-N, isolated from a case of disseminated coccidioidomycosis and 200-S, a soil isolate.

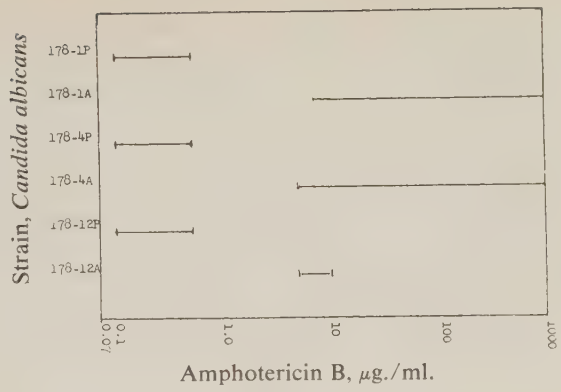
*Method of Inducing Resistance.* Graded amounts of amphotericin B in 0.1 ml. were added to 1.8 ml. of Mycophil broth in 0.5 oz. perfume bottles. The bottles were inoculated with 0.1 ml. of a suspension of *C. albicans* (112,000 blastospores/ml.) or 0.1 ml. of a 20 per cent ground suspension of *C. immitis*. The parent strains were kept under oil on agar in the refrigerator. The flasks were incubated for 24 to 72 hours at room temperature in a Kahn-type shaker. Growth in the flasks containing the largest amount of amphotericin B that allowed at least 50 per cent growth as compared to the control was used for the next series of transfers.

*Method of Comparing Sensitivity.* The perfume bottles were inoculated as described. All experiments were done in duplicate. Controls consisted of: (1) Medium 1.9 ml. plus 0.1 ml. of cells. Two of these were placed on the shaker as growth controls and one was placed in the refrigerator at 5 C. as suspension control. (2) In the cases where more than 200  $\mu\text{g.}/\text{ml.}$  of amphotericin B were used, flasks containing amphotericin B, medium, and cells were kept at 5 C. as a color and cell suspension control.

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FIG. 1. Zones of partial inhibition of *Candida albicans* caused by amphotericin B. P after the strain number indicates the parent strain from which the adapted (A) strain was derived.



The flasks were read at the end of 24 hours' incubation at room temperature on a Kahn-type shaker. The growth in the bottles was compared visually to the suspension and growth controls and recorded as growth, no growth, or inhibited growth, in those cases in which the turbidity lies between that of the growth and the suspension controls.

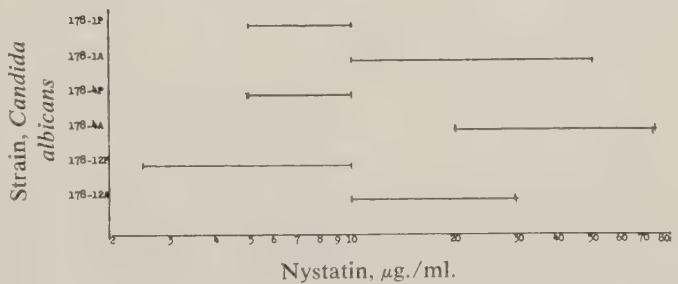
### RESULTS

Three strains of *C. albicans* and two strains of *C. immitis* were made more resistant to amphotericin B by continuous transfer in media containing increased increments of this antibiotic. In the case of *C. albicans* strains, about 10 transfers were needed to obtain about a three- to fivefold increase in resistance. Once this point was reached, the induced resistance proceeded rapidly. When these experiments were performed, 58 such transfers had taken place. Strains 178-1P and 178-4P were partially inhibited by 0.1 µg./ml. and completely inhibited by 0.5 µg./ml. of amphotericin B, before the induced resistance process began (fig. 1). After 58 transfers, these strains, designated as 178-1A and 178-4A, were partially inhibited by 7.5 and 5.0 µg./ml. respectively but were not completely inhibited by 1000 µg./ml. of amphotericin B. The resistant strains were slightly different morphologically in that they had a higher percentage of elongated cells and they tended to clump and settle out much more rapidly than the parent strains.

One of the resistant strains, 178-4A, was passed rapidly through 10 transfers, 1 loopful in 10 ml. of Mycophil broth, which had no amphotericin B, and then compared to its resistant parent. No differences in sensitivity to amphotericin B were detected, suggesting that this is a mutagenic selection rather than an adaptation process.

Since the better antifungal agents are polyene antibiotics, it was deemed of interest to determine if cross resistance existed between amphotericin B resistant cells and

FIG. 2. Zones of partial inhibition of *Candida albicans* caused by nystatin.



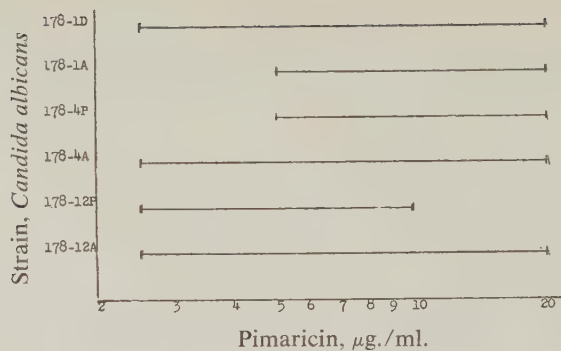


FIG. 3. Zones of partial inhibition of *Candida albicans* caused by pimaricin.

other polyene antibiotics. The *C. albicans* strains were compared using nystatin and pimaricin. Results in figures 2 and 3 indicate that *C. albicans* cells resistant to high concentrations of amphotericin B are also more resistant to nystatin. With pimaricin, cross resistance was not evident.

In similar experiments with two strains of *C. immitis*, it was possible to develop a tenfold increase in resistance to amphotericin B (based on the partial inhibition endpoint) as seen in figure 4. These two resistant strains were also more resistant to nystatin. The results with pimaricin are equivocal as the resistant strains showed an increase in the scope of the partial inhibition zone extending into the no inhibition zone as well as the inhibition zone.

#### DISCUSSION

It was found that it was possible to develop strains of *C. albicans* and *C. immitis* that are resistant to amphotericin B. In the case of *C. albicans*, two of the three strains were not completely inhibited by 100 μg./ml. with partial inhibition exhibited by 5 to 7.5 μg./ml. of this antibiotic. Littman et al<sup>2</sup> were able to produce strains of *C. tropicalis*, *C. krusei*, and *C. guillermoidii*, but they were unable to do so with *C. albicans*, the more important pathogen in this group. It appears that in their attempts to develop a resistant strain, they transferred cells from the high end of the

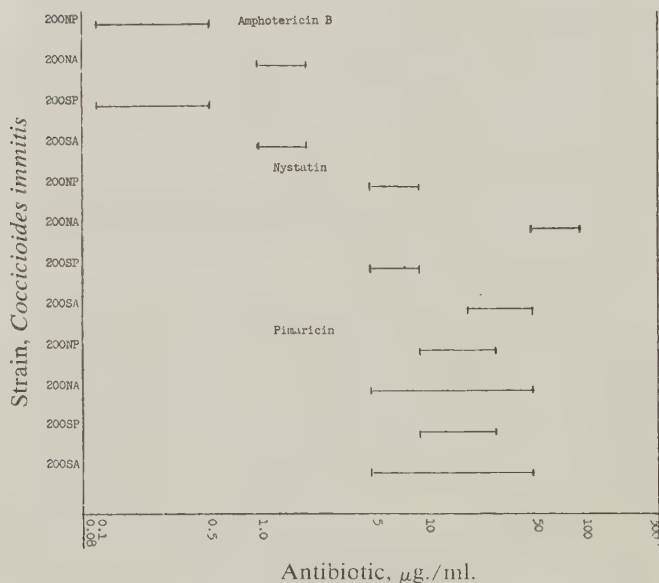


FIG. 4. Zones of partial inhibition of *Coccidioides immitis* caused by amphotericin B, nystatin, and pimaricin.

partial inhibition zone rather than the lower. In doing so, the cells had little opportunity to go through many generations prior to transfer to a higher level of the antibiotic than had they begun at a lower level.

Although the two cultures of *C. immitis* that were used in these studies did not develop the high degree of resistance that the *C. albicans* strains did, they did obtain a tenfold degree of resistance, which is of practical importance due to the fact that it is difficult to obtain high levels of amphotericin B in animal systems.

Both organisms showed cross resistance to nystatin, confirming the results of Littman et al<sup>2</sup> with other *Candida* species, but not to pimarin. Since nystatin is rarely used with *C. immitis* infections, this cross reaction does not assume the importance that it does with *C. albicans* where both antibiotics are of therapeutic value. Since this development of resistance appears to be of the mutational type rather than of the adaptational, these resistance forms assume greater importance especially should other polyene antibiotics be widely used. In any event, this whole problem should be expanded to include other organisms that cause deep-seated mycoses.

#### SUMMARY

Contrary to prior reports, it is possible to develop lines of *C. albicans* that are resistant to amphotericin B. Two strains of *C. immitis* were also made resistant to this antibiotic. These amphotericin B resistant strains were also resistant to nystatin, but were not resistant to pimarin. Since both nystatin and amphotericin B are used for the therapy of candidiasis, clinicians should be aware of the fact that resistance to amphotericin B can occur and that these strains are also resistant to nystatin.

#### ACKNOWLEDGMENT

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#### BIBLIOGRAPHY

1. DONOVICK, R.; PANSY, F. E.; STOUT, H. A.; STANDER, H.; WEINSTEIN, M. J., AND GOLD, W.: Some *in vitro* characteristics of nystatin (mycostatin). *In: Therapy of Fungus Diseases*, Boston, Mass., Little, Brown & Co., 1955, pp. 176-185.
2. LITTMAN, M. L.; PISANO, M. A., AND LANCASTER, R. M.: Induced resistance of *Candida* species to nystatin and amphotericin B. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc. 1958; pp. 981-987.
3. McNALL, E. G.; HALDE, C.; NEWCOMER, V. D., AND STERNBERG, T. H.: A biological assay for the determination of amphotericin A and B in biological fluids. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc. 1958; pp. 131-136.
4. STOUT, H. A., AND PAGANO, J. F.: Resistance studies with nystatin. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc. 1956; pp. 704-710.

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The activities of the majority of the presently known antimicrobial compounds are influenced by specific metallic ions.<sup>1</sup> Oddly enough, although bacitracin has been used clinically since 1947, no comprehensive studies of the effects of metallic ions or metal binding agents on the growth-suppressing or growth-promoting activities of this drug have been published. The in vitro antibacterial action of bacitracin has been briefly reported to be slightly enhanced by cobalt<sup>2</sup> but to be neither enhanced nor depressed by zinc.<sup>3</sup> However, the presence of an equimolar concentration of zinc in pharmaceutical preparations of bacitracin increases the stability of the drug to heat and prolonged storage.<sup>3</sup> In contrast to zinc, the presence of copper in as little as one-seventeenth the molar concentration of bacitracin at pH 8.4, but not at pH 7.0, results in almost complete loss of activity of the antibiotic within four hours.<sup>4</sup> The drug has also been reported to be inactivated by salts of heavy metals lower in the electromotive series than zinc and to be partially inactivated by the metal binding agent, dimercaprol (BAL).<sup>5</sup> To extend our knowledge of the effect of metallic ions and metal binding agents on the antibacterial action of bacitracin, the following in vitro study was undertaken.

## MATERIALS AND METHODS

*Bacterial Strains and Culture Media.* The test bacteria and their sensitivity to bacitracin (minimum inhibitory concentration in nutrient agar) consisted of a stock culture strain each of *Staphylococcus aureus*, 2.0  $\mu\text{g.}/\text{ml.}$ ; *Bacillus subtilis*, 80  $\mu\text{g.}/\text{ml.}$ ; and *Escherichia coli*, 750  $\mu\text{g.}/\text{ml.}$  The culture media employed in the study consisted of nutrient broth, 0.5 per cent polypeptone (BBL) and 0.3 per cent beef extract (Difco) dissolved in distilled water and nutrient agar, 2.0 per cent flake agar (BBL) dissolved in nutrient broth.

*Bacitracin.* The drug (U.S.P., 65 units/mg.) was purchased from the California Foundation for Biochemical Research; the lot number is 102175. The antibiotic was dissolved in demineralized water and filtered through sintered glass. The antibiotic solutions, which were prepared freshly each week, were stored at  $-10^{\circ}\text{C.}$  when not in use.

*Methods of Testing the Effect of Metallic Ions and Metal Binding Agents on the Activity of Bacitracin.* The effect of the test substances on the antibacterial activity of the drug was observed first by the double-gradient plate method.<sup>6</sup> In this method, the sterile metallic salt or metal binding agent is included in the first or lower layer of the agar medium (which is solidified while the plate is in an inclined position), and the drug is incorporated in the second or upper layer (solidified while the plate is in a level position). Single-gradient plates containing either the drug or the particular test substance are routinely included as controls. The maximum, nontoxic concentration of each test substance for each bacterial strain is determined in preliminary trials with single-gradient plates. These concentrations are then used in the double-gradient experiments.

Each of the solutions of the test substances, before addition to the medium, is

neutralized with sodium hydroxide or hydrochloric acid, and the pH reactions of duplicate uninoculated double-gradient plates containing each substance are checked with bromthymol blue, to make certain that the entire surface of each experimental plate has a pH value of 7.0. Approximately  $10^3$  viable cells of the various test organisms are spread evenly over each agar surface of the experimental plates, and the extent of visible growth is observed after 24 hours of incubation at 37C. The metallic salts and metal binding agents employed in the present study are listed in table 1.

To confirm by other methods the activity of those substances that affect bacitracin, experiments were then performed with *Staph. aureus* and *B. subtilis* in non-gradient nutrient agar plates and in shaken nutrient broth flasks. Various plates and flasks were supplemented with appropriate concentrations of the drug and test substances and were then inoculated with cells of the bacterial test strains. The extent of growth was recorded after 24 hours at 37C. Inoculated, unsupplemented media in plates and flasks were routinely included and served as the sources of inocula for the subsequent day's experiments.

RESULTS

The observations made with the double-gradient plates clearly indicate that, with each of the test organisms, the antibacterial activity of bacitracin is strongly enhanced by salts of zinc and is strongly suppressed by salts of ethylenediaminetetraacetate and cyclohexanediaminetetraacetate (EDTA and CDTA). Moderate enhancement is obtained with low concentrations of salts of cadmium and mercury; perhaps these salts in larger amounts would be as active as salts of zinc, but the toxicity of the ions of cadmium and mercury precludes a testing of this hypothesis. Slight enhancement, with *Staph. aureus* and *B. subtilis*, is obtained with salts of aluminum and cobalt in concentrations equal to the salts of zinc. Salts of no other metallic ions or metal binding agents affect the activity of the drug.

The results obtained with zinc and with EDTA are summarized in figure 1. It may be noted that differences of as much as four hundred fifty fold in the antibacterial activity of bacitracin can be obtained merely by including in nutrient agar concentrations of salts of zinc or EDTA which, in themselves, have no apparent effect on the growth of the test organisms. With the double-gradient plate method, it is possible to demonstrate the synergistic effect of zinc and bacitracin in a single Petri plate. A diagrammatic representation of this demonstration is given in figure 2. It may be observed in the fourth Petri plate that growth is inhibited in the region containing a moderate amount of bacitracin and zinc. It is significant that growth occurs in the region containing the maximum amount of bacitracin but insufficient

TABLE I  
Individual Test Substances Added to Lower Layers  
of Double-Gradient Nutrient Agar Plates

Metallic salts	Metal binding agents
Nitrates of Na <sup>+</sup> , Ag <sup>+</sup> , Ca <sup>++</sup> , Sr <sup>++</sup> , Ba <sup>++</sup> , Co <sup>++</sup> , Pb <sup>++</sup> , Hg <sup>++</sup> , Al <sup>+++</sup>	Sodium salts of phosphate, tartrate, and citrate
Sulfates of Na <sup>+</sup> , Mg <sup>++</sup> , Mn <sup>++</sup> , Fe <sup>++</sup> , Ni <sup>++</sup> , Cu <sup>++</sup> , Zn <sup>++</sup> , Cd <sup>++</sup> , Al <sup>+++</sup>	Disodium salts of ethylenediamine-tetraacetate (EDTA) and cyclo-hexanediaminetetraacetate (CDTA)
Chlorides of Na <sup>+</sup> , Mg <sup>++</sup> , Zn <sup>++</sup> , Sn <sup>++</sup> , Hg <sup>++</sup> , Fe <sup>+++</sup> , Sb <sup>+++</sup>	
Sodium salts of Se <sup>-</sup> , MoO <sub>4</sub> <sup>-</sup> , WoO <sub>4</sub> <sup>-</sup>	

zinc, as well as in the region containing the maximum concentration of zinc but insufficient bacitracin.

In subsequent double-gradient plate experiments, EDTA was combined in the lower layer with excess molar concentrations of salts of each of the 21 metallic ions. The ability of EDTA to suppress bacitracin was found to be inhibited only by salts of zinc, cadmium, mercury, aluminum, or cobalt. Salts of other metallic ions that combine with EDTA at pH 7.0 are unable to neutralize the action of the metal binding agent. When salts of zinc and EDTA are combined in an equimolar ratio of metallic ion to metal binding agent, bacitracin is neither enhanced nor suppressed. None of the 20 other metallic ions are able to affect the ability of ions of zinc to enhance the antibiotic.

The results of the quantitative tests in single layer (nongradient) nutrient agar plates with salts of zinc and with EDTA confirmed the observations made with *Staph. aureus* and *B. subtilis* with double-gradient plates. Similar results were obtained in shaken nutrient broth. In a different series of experiments, it was found that concentrations of bacitracin up to 5000 times the minimal inhibitory concentration can prevent the growth of *Staph. aureus* in nutrient agar in the absence of added zinc. Higher concentrations were not tested.

#### DISCUSSION

As is true of the majority of antimicrobial compounds, the antibacterial activity of bacitracin has been observed to fluctuate with the type and composition of the

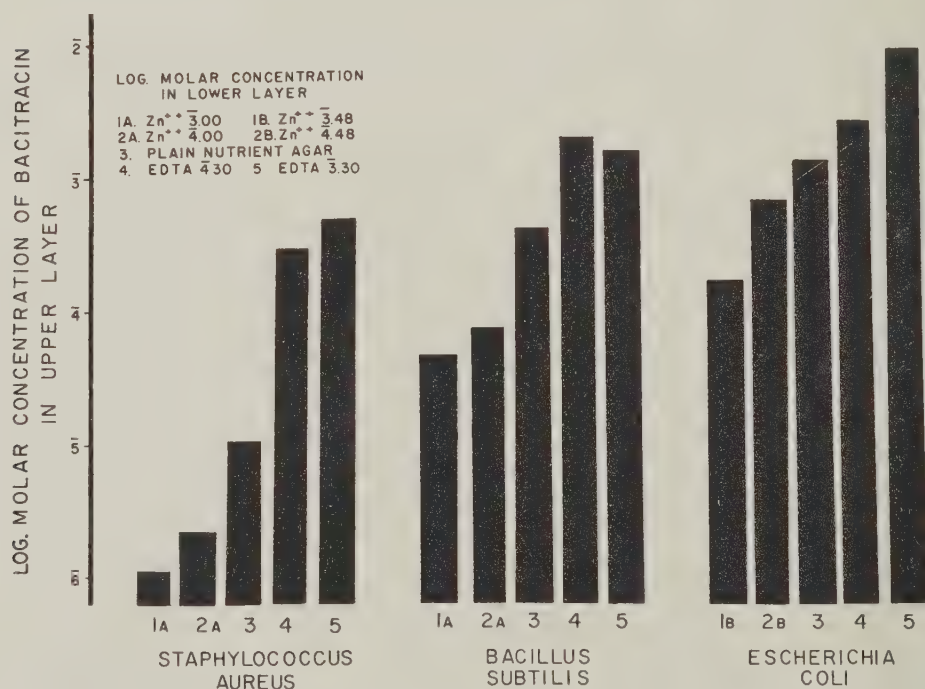
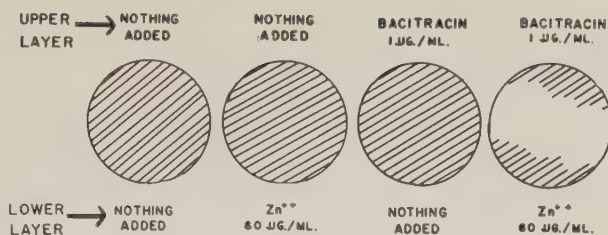


FIG. 1. Effect of zinc and EDTA on the antibacterial activity of bacitracin. The height of each bar indicates the concentration of bacitracin that permits growth on 50 per cent of the agar surface when the drug is incorporated in the upper layer and either zinc chloride or EDTA is included in the lower layer of double-gradient nutrient agar plates. In the concentrations used, neither zinc chloride nor EDTA affect bacterial growth. The molar concentration of bacitracin is calculated on the basis of the proposed molecular weight of 1421.<sup>7</sup>

FIG. 2. Extent of growth of *Staphylococcus aureus* on double gradient plates containing bacitracin and/or zinc chloride. The shaded areas represent visible growth after 24 hours at 37 C.



complex medium employed.<sup>8</sup> Inasmuch as the concentrations of ions of zinc in the ingredients of such media is quite variable,<sup>9</sup> the reason for the fluctuation of bacitracin potency is now apparent. In eight brands of peptone tested by spectrographic analysis, the amount of zinc varied from 11 to 300 p.p.m.<sup>9</sup> Moreover, some samples within a single brand were not consistent in their content of metallic ions. Two brands of peptone widely used in the United States, Difco proteose peptone and BBL polypeptone, contain, respectively, 44 and 110 p.p.m. of zinc.<sup>10</sup> Unfortunately, metallic ion analyses are not available for peptones supplied for bacteriological use by Wilson and Co., Armour and Co., or Nutritional Biochemicals Corp.<sup>11</sup>

Another potential source of variation in the concentration of zinc in the environment is that associated with the particular lot of bacitracin. Preparations of high purity are often not so stable as less pure lots,<sup>12</sup> possibly because some of the associated zinc has been removed by the purification procedure. In a study of contamination by heavy metals of clinically used antibiotics, Selzer observed that 13 samples of bacitracin contained from 15 to 110 p.p.m. of such metals.<sup>13</sup> It is not known how much of this contamination consisted of zinc. The author stated that such a wide variation (typical of no other drug examined) in metal contamination of bacitracin might be reduced by appropriate Food and Drug Administration control. Perhaps the concentration of zinc should be deliberately increased to obtain maximum chemotherapeutic activity. In vivo tests are obviously needed to ascertain if the desirable pharmacological characteristics of bacitracin can be enhanced by zinc. As stated in the introduction, it is well established that this metallic ion confers thermal and storage stability on the drug, but it has been previously believed that the antibacterial action of the peptide is not significantly affected by zinc.<sup>3</sup>

The ability of zinc to confer stability on bacitracin undoubtedly indicates that the metallic ion can combine chemically with the antibiotic. Perhaps the situation with bacitracin is analogous to that of insulin, which has been postulated to interact with zinc at the surface of the mammalian cell.<sup>14</sup> It has long been known that zinc forms part of the crystal lattice of insulin, and it has recently been shown that EDTA suppresses certain biochemical activities of the protein.<sup>14</sup>

There exists a noticeable lack of fundamental knowledge of the mechanism of antibacterial action of bacitracin. With the exception of one brief paper,<sup>15</sup> there are no published reports on biochemical activities of bacteriostatic concentrations of the drug. In view of the results of the present study, the effect of bacitracin on enzymatic systems that require zinc and on enzymatic systems that are inhibited by zinc should be investigated.

Albert has cited numerous examples of the biological activation of organic compounds by metallic ions.<sup>16</sup> However, the phenomenon described by Albert of "concentration quenching" (previously called the "zone phenomenon" by Eagle and Musselman<sup>17</sup>) could not be demonstrated in the present study with bacitracin. Perhaps zinc is not needed for the diffusion of the drug into the cells, or perhaps there is sufficient zinc in the samples of the antibiotic or in the cell walls of the bacteria

to permit large quantities of the drug to be as active as small concentrations in suppressing growth of the organisms.

Bacitracin has been shown to stimulate growth of young poultry<sup>18</sup> and plants.<sup>19</sup> Inasmuch as the intestinal microorganisms of the birds remained unaltered and the plants were grown aseptically, it is clear that the growth-promoting action of the drug can be independent of any effects on microbial flora of young animals and plants. Rather, in common with other metal binding substances, bacitracin can probably stimulate growth by controlling the diffusion and assimilation of metallic ions into the tissues and cells of the plants and animals. It would be of considerable interest to determine if the growth-promoting action of the drug would be suppressed if the diet of the macroorganisms were enriched with excess zinc or completely deprived of zinc.

It is apparent that a considerable amount of work is needed to ascertain the precise role of zinc in the antibacterial and nutritional activities of bacitracin. However, it is now clear that all persons—biologists, chemists, pharmacologists, manufacturers, pharmacists, and physicians—who wish successfully to study, produce, or use this drug must be aware not only of the concentration of zinc but also of the agents that can bind zinc in the biological systems with which they are working.

#### SUMMARY

The effect of 21 individual metallic ions and 5 metal binding agents on the antibacterial activity in vitro of bacitracin was examined. With each test strain, strong drug enhancement was exhibited by salts of zinc and moderate enhancement by salts of cadmium and mercury. Slight drug enhancement, with the gram-positive strains, was exhibited by salts of aluminum and cobalt. Ethylenediaminetetraacetate and cyclohexanediaminetetraacetate strongly suppressed bacitracin. It is suggested that, in addition to enhancing the thermal and storage stability of the drug, zinc is also associated with the mechanisms of antibacterial and nutritional action of bacitracin.

#### ACKNOWLEDGMENTS

It is a pleasure to acknowledge the financial support of the National Science Foundation and the excellent technical assistance of Judith Irene Brooks, Joan Burdsall Schmidt, and Lois Ann Francis. The sample of CDTA used in this study was supplied by Geigy Chemical Corporation through the courtesy of Dr. H. W. Zussman.

#### BIBLIOGRAPHY

1. WEINBERG, E. D.: The mutual effects of antimicrobial compounds and metallic cations, *Bact. Rev.* 21:46, 1957.
2. TRACE, J. C., AND EDDS, G. T.: The influence of cobalt on the action of antibiotics, *Am. J. Vet. Research* 15:639, 1954.
3. GROSS, H. M.: Zinc bacitracin in pharmaceutical preparations, *Drug and Cosmetic Ind.* 75:612, 1954.
4. SHARP, V. E.; ARRIAGADA, A.; NEWTON, G. G. F., AND ABRAHAM, E. P.: Ayfivin: Extraction, purification, and chemical properties, *Brit. J. Exper. Path.* 30:444, 1949.
5. ANKER, H. S.; JOHNSON, B. A.; GOLDBERG, J., AND MELENEY, F. L.: Bacitracin: Methods of production, concentration, and partial purification, with a summary of the chemical properties of crude bacitracin, *J. Bact.* 55:249, 1948.
6. WEINBERG, E. D.: Double-gradient agar plates, *Science* 125:196, 1957.
7. HAUSMANN, W.; WEISIGER, J. R., AND CRAIG, L. C.: Bacitracin A. Further studies on the composition, *J. Am. Chem. Soc.* 77:721, 1955.

8. COHEN, J. O., AND PRATT, D. B.: The effect of culture media components on the sensitivity of *Micrococcus pyogenes* var. *aureus* to bacitracin, *Antib. & Chemo.* 6:475, 1956.
9. SYKES, G.: Constituents of Bacteriological Culture Media, Cambridge, Cambridge University Press, 1956, p. 3.
10. CHRISTENSON, C. W., AND VERA, H. D.: Personal communications.
11. BURNETT, R. S.; KING, E. G., AND MANN, S. M.: Personal communications.
12. SPECTOR, W. S. (Ed.): Bacitracins. *In: Handbook of Toxicology, Volume II: Antibiotics.* Philadelphia, W. B. Saunders Co., 1957, p. 29.
13. SELZER, G. B.: Heavy metals in antibiotics, *Antib. & Chemo.* 6:498, 1956.
14. KRAHL, M. E.: Speculations on the action of insulin, with a note on other hypoglycemic agents, *Perspectives in Biol. and Med.* 1:69-96, 1957.
15. PAINE, T. F., JR.: The similarity in action of bacitracin and penicillin on the *Staphylococcus*, *J. Bact.* 61:259, 1951.
16. ALBERT, A.: Metal-binding agents in chemotherapy: The activation of metals by chelation. *In: The Strategy of Chemotherapy*, Cambridge, Cambridge University Press, 1958, p. 112.
17. EAGLE, H., AND MUSSELMAN, A. D.: The rate of bactericidal action of penicillin in vitro as a function of its concentration, and its paradoxically reduced activity at high concentrations against certain organisms, *J. Exper. Med.* 88:99, 1948.
18. ELAM, J. F.; GEE, L. L., AND COUCH, J. R.: Function and metabolic significance of penicillin and bacitracin in the chick, *Proc. Soc. Exper. Biol. & Med.* 78:832, 1951.
19. NICKELL, L. G., AND FINLAY, A. C.: Antibiotics and their effects on plant growth, *Agric. and Food Chem.* 2:178, 1954.

# Low-level, Long-term Feeding of Chlortetracycline and the Emergence of Antibiotic-Resistant Enteric Bacteria

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In several recent reports<sup>1-3</sup> it was indicated that nonmedical use of antibiotics was on the increase and that the area of low-level, long-term intake of antibiotics required investigation, particularly with regard to the emergence of resistant bacteria. In 1957 it was reported<sup>4</sup> that feeding of streptomycin to experimental animals at low levels (20 to 40  $\mu\text{g.}/\text{animal}/\text{day}$ ) for several months induced the emergence of streptomycin-resistant coliform bacteria. Since the percentage of resistant bacteria occurred at a significant rate and the level of resistance was as high as 10,000  $\mu\text{g.}/\text{ml.}$ , it was concluded that a certain amount of public health hazard was potentiated by low-level chronic intake of streptomycin.

In the present study an investigation was organized to acquire similar data for the tetracycline antibiotics, since clinical therapeutic experience has indicated tetracycline antibiotics may be less of a problem than streptomycin when considering emergence of resistance.

This paper therefore reports the effect of low-level, long-term chlortetracycline on the emergence of resistance of enteric flora. In addition, it compares these results to similar streptomycin studies and also illustrates comparative chlortetracycline antibiotic serum levels obtained by ingestion of chlortetracycline residue in treated food and as a therapeutic antibiotic.

## MATERIALS AND METHODS

Feeding of chlortetracycline to experimental animals was carried out essentially the same as previously reported for streptomycin.<sup>4</sup> Two hundred white Swiss mice, (20 groups, 10/group), were fed the antibiotic at 0-0, 20 and 40  $\mu\text{g.}/\text{day}$ . Pooled fecal samples were obtained, weighed, homogenized, and then cultured for coliforms and other enterics on MacConkey agar containing 0, 10, 50, 100, 500, and 1000  $\mu\text{g.}/\text{chlortetracycline}/\text{ml.}$

Thus determinations were made of the effect of chlortetracycline on total enteric count, variation of predominating organism and emergence of resistance, over a seven months' feeding period.

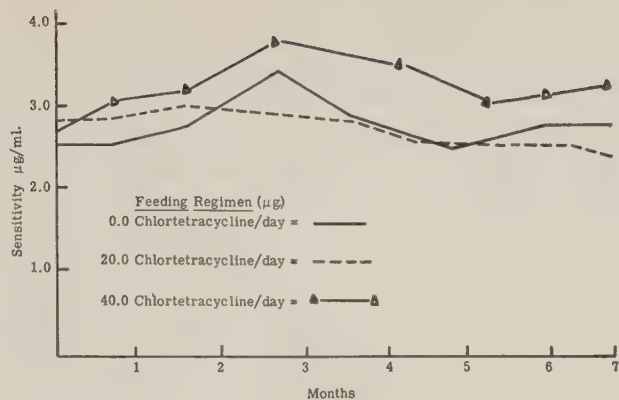
In a related study on chlortetracycline blood levels, 10 rabbits (2.0 to 2.5 Kg.) were fed spinach that was treated with chlortetracycline for preservative purposes. This spinach had a residue such that rabbits receiving 40 Gm. of spinach per day were ingesting 250  $\mu\text{g.}$  of chlortetracycline residue as determined by microbiological assay.<sup>5</sup>

These rabbits were maintained on this routine during a 12 weeks' period during which time blood serum assays following Grove and Randall's technique<sup>5</sup> were carried out once each week. In order to compare these serum levels with those obtained from therapeutic doses of chlortetracycline, another series of rabbits were fed therapeutic dose (33 mg.) of chlortetracycline and assayed similarly.

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FIG. 1. The chlortetracycline sensitivity of coliform bacteria from mice fed long-term, low-level antibiotics is shown.



## RESULTS

In the preliminary study, testing sensitivity of hundreds of mouse coliform bacteria to chlortetracycline it was determined that they were inhibited, on the average, by 2.8 µg. of the antibiotic. However, sensitivity ranged from 2.0 to 4.0 µg. at the extremes. Noncoliform organisms (lactose-negative bacteria) were inhibited by 5.0 µg. chlortetracycline. These organisms were ultimately shown to be mostly *Proteus* sp. with a few *Pseudomonas* sp.

For comparison with previous studies on streptomycin, it was established that coliform and noncoliform organisms would be considered resistant if they required 10 µg. of chlortetracycline for minimum inhibition.

Figure 1 compares the chlortetracycline sensitivity of coliforms from mice fed 0, 20, and 40 µg. daily chlortetracycline during seven months along with normal antibiotic-free mouse pellets. It can be observed that throughout this period no significant resistant coliforms appeared. Very little variation in sensitivity occurred in the coliform flora of these mice getting chlortetracycline. These determinations were made with the gradient plate technique<sup>6</sup> and confirmed by incorporating chlortetracycline in MacConkey's agar at 10 µg./ml. with subsequent failure of coliform bacteria to grow on the plates. It should be noted here that antibiotic levels incorporated in the media were calculated by assay to consider antibiotic breakdown in this media.

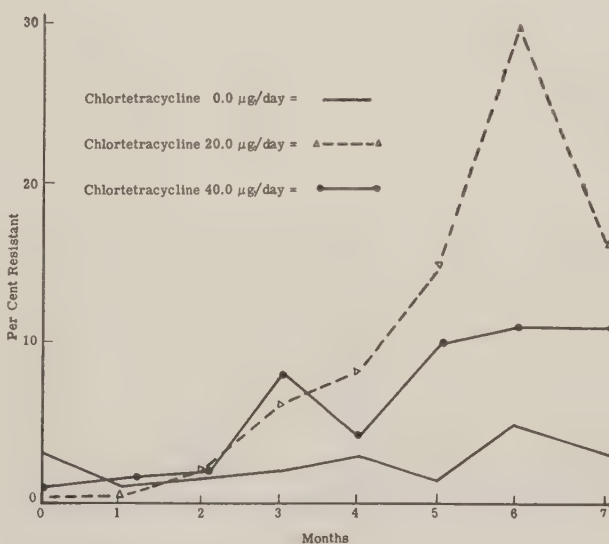
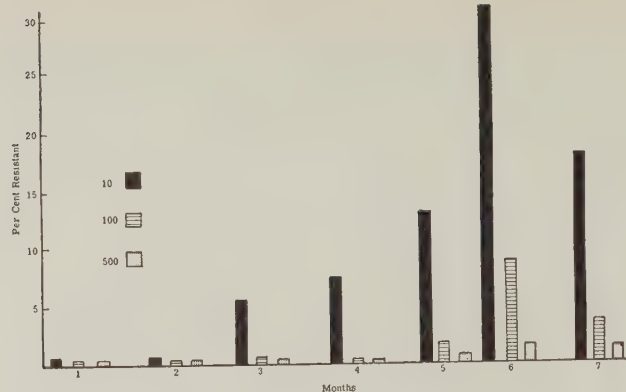


FIG. 2. The chlortetracycline resistance (resistant to 10 µg./ml.) of lactose-negative enterics (*Proteus-Pseudomonas*) from mice fed long-term, low-level chlortetracycline is illustrated.



Data on noncoliform organisms (fig. 2) indicate that they do develop resistance on exposure to long-term, low-level chlortetracycline. These organisms, identified mainly as *Proteus*, begin to show resistance at the third to fourth month, develop to their highest level at the sixth month, and then decrease. Higher resistance is observed from mice fed 20 µg./day than from mice fed 40 µg./day.

Analysis of the degree of resistance by the noncoliform bacteria is seen in figure 3. It appears that major resistance is limited to 10 µg./ml.; at 100 µg. and at 500 µg. only a small percentage of noncoliforms were showing resistance. None of the organisms in the study were resistant to 1000 µg. of chlortetracycline.

Direct comparison of maximum resistance level between streptomycin-resistant enterics and chlortetracycline-resistant enterics is described in figure 4. Feeding low levels of these antibiotics is seen to result in greater percentage of resistance to higher levels from streptomycin. Feeding chlortetracycline under these conditions one observes that not more than 5 per cent of resistance is at 500 µg. levels while for streptomycin 5 to 40 per cent resistance is at the level of 10,000 µg.

Blood levels from feeding daily low-level chlortetracycline were determined for rabbits ingesting spinach treated with antibiotics. Figure 5 illustrates that the level attained after four to five weeks feeding was approximately .020 µg./ml. and never increased though feeding of 250 µg. chlortetracycline in spinach was continued daily for 12 weeks. The assay sensitivity was 0.005 µg./ml. chlortetracycline. When compared this level is about 100 times less than that accrued by feeding a rabbit a daily therapeutic dose (33 mg.) for a similar period.

In addition to these results it was also apparent from feeding long-term, low-level chlortetracycline to mice that no drastic change occurred in total bacterial count, in predominating flora, or in general well-being of the animals.

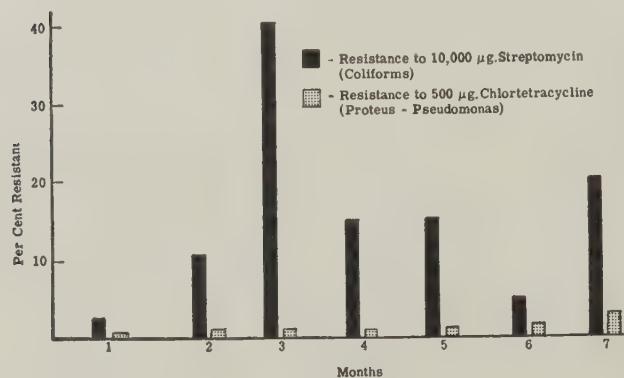
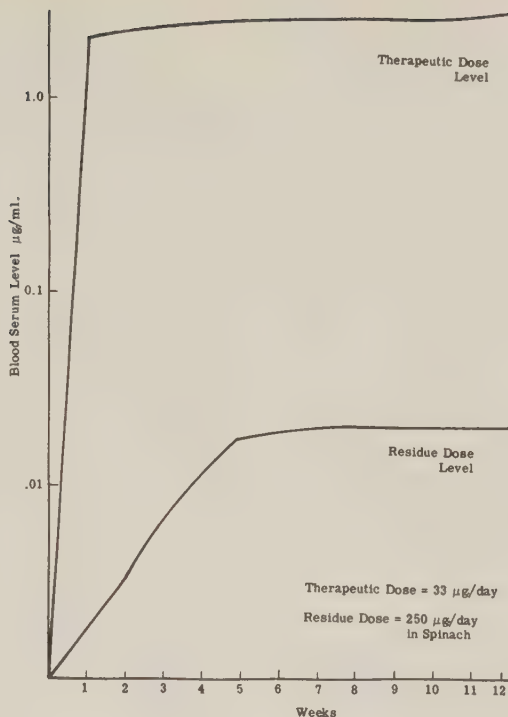


FIG. 5. A comparison of antibiotic serum levels of rabbits fed residue chlortetracycline in food and as therapeutic dose is given. Triplicate assays of 10 rabbits were made.



#### DISCUSSION

These results undoubtedly reiterate that emergence of resistance to antibiotics depends on the dosage level, the type of bacteria, and the specific antibiotic.

Chlortetracycline had no effect on inducing resistance of coliform bacteria, whereas under similar conditions streptomycin induced resistance.<sup>4</sup> The 20 µg. level of chlortetracycline brought about some resistance in noncoliform organisms, but it was not so great a percentage nor were the levels of resistance as high as induced by streptomycin. In all cases (chlortetracycline and streptomycin) the maximum resistance appears to be transitory with peaks four to six months after feeding followed by decline in resistance.

Chlortetracycline blood level studies indicate that feeding low levels does not result in any cumulative serum level which even approach therapeutic levels. The maximum levels attained are always such a small fraction of a microgram that they would undoubtedly have little or no effect on inducing chlortetracycline resistance of normal or pathogenic flora.

For some time now it has been obvious that the antibiotics could play a greater role in food treatment by preventing bacterial plant disease *in vivo* or by increasing shelf life and storageability. For safety, it is demanded that we first investigate potential hazards, i.e., emergence of resistance, sensitization of persons, and cumulative toxicity. On the basis of animal study to date on the potential hazard of emergence of antibiotic resistant bacteria, it would seem that little if any risk is involved with use of the tetracycline antibiotics.

The over-all percentage of organisms becoming resistant is limited and the duration of resistance is not permanent. Confirmation of these facts with human adult volunteers would settle the question.

## CONCLUSIONS

On the basis of a two year study feeding low-level (20-40  $\mu\text{g.}/\text{animal}/\text{day}$ ), long-term antibiotics (streptomycin, chlortetracycline) to animals in water and as residues in treated food, the following conclusions relative to emergence of resistance of intestinal flora are made.

1. Five to 40 per cent of all coliforms emerge resistant to levels of 10,000  $\mu\text{g.}/\text{ml.}$  following streptomycin at long-term, low-level feeding. Noncoliform lactose-negative organisms do not develop this resistance pattern.
2. Chlortetracycline does not cause detectable emergence of resistance by coliform bacteria. Some resistance does occur with lactose-negative enterics (*Proteus-Pseudomonas*). However, these levels of resistance are minimal and percentage of organisms exhibiting resistance is low when compared to the streptomycin results.
3. Long-term, low-level feeding of chlortetracycline does not result in antibiotic blood serum levels which approach therapeutic levels. Daily feeding of low-levels of chlortetracycline in food for 12 weeks resulted in antibiotic serum levels that were 1/100 of those attained by corresponding therapeutic dose.
4. It would seem that public health problems, such as emergence of antibiotic resistance anticipated by widespread nontherapeutic usage of antibiotics, are less likely with tetracycline antibiotics than with streptomycin. Attempts are underway to confirm this by studies on adult human volunteers.

## BIBLIOGRAPHY

1. READ, B.; GOLDBERG, H. S., AND GOODMAN, R. N.: The effect of long-term low-level feeding of streptomycin on the streptomycin resistance of normal flora, *Bact. Proc.* 1957, p. 21.
2. ANON.: First International Symposium Antibiotics in Agriculture: National Research Council Publ. no. 397, 1956.
3. LOGUE, J. T.; GOLDBERG, H. S., AND GOODMAN, R. N.: The public health significance of antibiotic residues in foods. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; pp. 333-335.
4. GOLDBERG, H. S.; READ, B. E., AND GOODMAN, R. N.: Studies on the emergence of streptomycin-resistant bacteria as a result of low-level long-term feeding of streptomycin. *In: Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958; pp. 144-148.
5. GROVE, D. C., AND RANDALL, W. A.: *Assay Methods of Antibiotics; A Laboratory Manual*. New York, Medical Encyclopedia, Inc., 1955.
6. BRAUDE, A. I.; BANISTER, J., AND WRIGHT, N.: Use of the gradient plate for routine clinical determinations of bacterial sensitivities to antibiotics. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955; p. 1133.

# Suppression of Growth of Fungal Microflora in the Conservation of Meat by Chlortetracycline

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The inhibition of growth of bacterial microflora in the conservation of meat and especially of poultry was successfully solved by the application of the tetracycline antibiotics; there remained, however, unsolved the problem of inhibition of the contamination by fungi, the overgrowth of which is often observed after the application of the tetracyclines.<sup>1,2,4-7</sup> Because of the practical importance of this problem, we studied the effects of combinations of chlortetracycline with other antimicrobial agents in order to broaden the scope of activity of the preparations to cover yeasts as well as bacteria.

## MATERIALS AND METHODS

The strains used for testing the activity of antibiotics and other antimicrobial agents were isolated from the surface of whole uneviscerated chickens that had been kept at a temperature of 25 C. for three days. The strains were isolated without any intentional selection. Our collection contained 150 cultures of bacteria and 70 cultures of yeasts. Filamentous fungi were not detected on our cultivation media (nutrient broth, malt and Czapek-Dox agar).

The antimicrobial activity was tested on a nutrient broth medium containing 1 per cent of glucose and 2.5 per cent of agar, having a pH 7.0. The antibiotics and antimicrobial agents were dissolved or suspended in a small volume of water and added to the medium at 45 C. shortly before the filling of the plates. The complex of chlortetracycline with benzalkonium chloride was dissolved in 0.1 *N* hydrochloric acid prior to its addition to the medium.

Activity was tested by the replica plating method.<sup>3</sup> The plates were point inoculated by about 40 cultures, and these were transferred after 48 hours by a velvet stamp to successively four to six plates with media containing the studied substances in appropriate concentrations. The control plate was the last to be stamped. Replica plating repeated up to seven times does not decrease the reliability of the test. The inoculated plates were inspected after 24 hours of cultivation at 28 C. The cultures, which on some rare occasions did not form colonies on the control plate, were not taken into account in the evaluation of antimicrobial agents.

## RESULTS

The results obtained with chlortetracycline hydrochloride, the complex of chlortetracycline with benzalkonium chloride, benzalkonium chloride, fungicidin, sorbic acid, and alcohol are given in figure 1. Chlortetracycline hydrochloride in a concentration of 50 p.p.m. inhibits growth of 73 per cent of cultures of bacteria and 0 per cent of cultures of yeasts. The complex of chlortetracycline with benzalkonium chloride inhibits 80 per cent of cultures of bacteria and 14 per cent of cultures of yeasts. The complex shows a higher activity compared with chlortetracycline hydrochloride even in a concentration of 10 and 100 p.p.m.

Benzalkonium chloride itself in a concentration of 5 p.p.m. inhibits 4.7 per cent

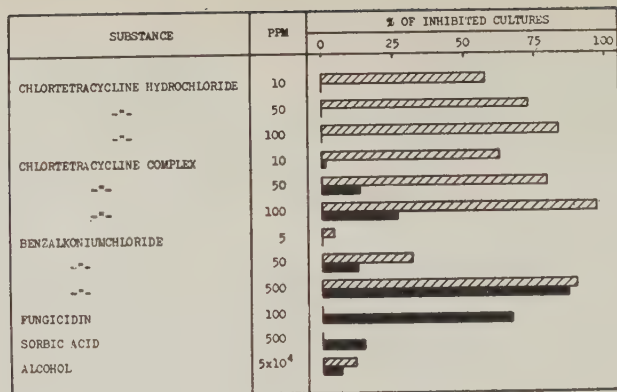


FIG. 1. Inhibition of bacteria and yeasts by some antibiotics and anti-microbial agents. [Hatched] bacteria; [Solid] yeasts.

of cultures of bacteria and none of cultures of yeasts, in a concentration of 50 p.p.m., 32 per cent cultures of bacteria and 13 per cent of cultures of yeasts, and in a concentration of 500 p.p.m., 90 per cent of cultures of bacteria and 87 per cent of cultures of yeasts.

Fungicidin in a concentration of 100 p.p.m. does not inhibit any bacteria from our collection, and it inhibits 67 per cent of our cultures of yeasts. Sorbic acid in a concentration of 500 p.p.m. inhibits growth of only 15 per cent of cultures of yeasts.

Ethyl alcohol in a concentration of 5 per cent inhibits, under our experimental conditions, 12 per cent of our cultures of bacteria and 7 per cent of our cultures of yeasts.

The effect of a mixture of chlortetracycline hydrochloride with fungicidin and of the mixture of the chlortetracycline benzalkonium chloride complex with fungicidin is shown in figure 2. The concentration of fungicidin was in all cases 100 p.p.m. At a concentration of 50 p.p.m. of the tetracycline, the mixture of the chlortetracycline and benzalkonium chloride complex with fungicidin inhibits 10 per cent more cultures of both bacteria and yeasts than the mixture of chlortetracycline hydrochloride with fungicidin. The higher activity of the mixture of chlortetracycline and benzalkonium chloride complex with fungicidin is evident also at a concentration of 10 p.p.m. of chlortetracycline, especially with yeasts.

In a further series of experiments we studied the effects of chlortetracycline hydrochloride, chlortetracycline benzalkonium chloride complex, and fungicidin in the presence of 5 per cent ethanol. The inhibition of yeasts under these conditions is given in figure 3. The influence of chlortetracycline hydrochloride and of the complex upon cultures of bacteria is not affected by the presence of 5 per cent of ethanol in the medium. A combination of fungicidin (50 to 100 p.p.m.) with 5 per cent of ethanol inhibits growth of 82 per cent of cultures of yeasts, i.e., substantially more than fungicidin alone (67 per cent at 100 p.p.m.). Maximum suppression of yeasts was obtained by the combination of the chlortetracycline complex (50 p.p.m.) with 5 per cent of ethanol, i.e., 93 per cent of inhibition. This degree of inhibition is not further increased by the addition of 100 p.p.m. of fungicidin. The concentration of

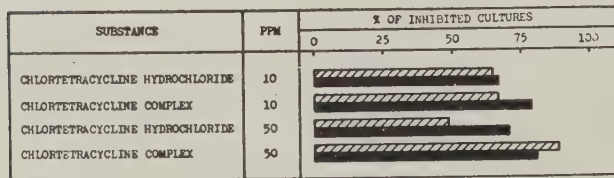
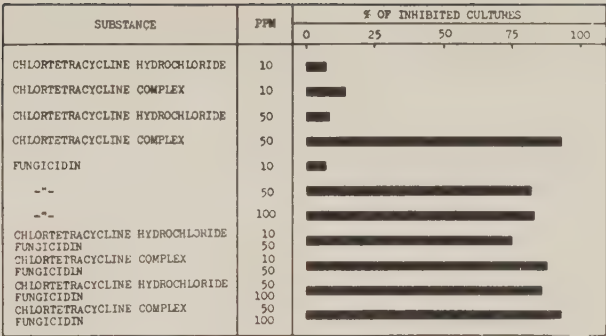


FIG. 2. Inhibition of bacteria and yeasts by chlortetracycline hydrochloride and by the chlortetracycline benzalkonium chloride complex in the presence of 100 p.p.m. of fungicidin. [Hatched] bacteria; [Solid] yeasts.

FIG. 3. Inhibition of yeasts by chlortetracycline hydrochloride, chlortetracycline benzalkonium chloride complex, fungicidin, and their combinations in the presence of 5 per cent ethanol.



5 per cent of ethanol was used in our experiments because of the fact that 10 per cent of ethanol inhibit practically 100 per cent of the tested cultures of both bacteria and yeasts.

### SUMMARY

The activity of mixtures of chlortetracycline with other antimicrobial agents against bacteria and yeasts isolated from the surface of uneviscerated chickens was studied in vitro. The complex of chlortetracycline with benzalkonium chloride shows higher activity against bacteria than chlortetracycline hydrochloride, and in addition it also inhibits yeasts. The mixture of the complex with fungicidin is more active against both bacteria and yeasts than the mixture of chlortetracycline hydrochloride with fungicidin. The activity of the chlortetracycline and benzalkonium chloride complex and of fungicidin against yeasts may be increased by the addition of a small amount of ethanol.

### BIBLIOGRAPHY

1. BARNES, E. M.: Should antibiotics be used as food preservatives? *Food Manufact.* 31:508-510, 1956.
2. BARNES, E. M.: New methods in food preservation. (a) Antibiotics, *Royal Soc. Health J.* 77:447-457, 1957.
3. LEDERBERG, J., AND LEDERBERG, E. M.: Replica plating and indirect selection of bacterial mutants, *J. Bact.* 63:399-406, 1952.
4. NJOKU-OBI, A. N.; SPENCER, J. V.; SAUTER, E. A., AND EKLUND, M. W.: A study of the fungal flora of spoiled chlortetracycline treated chicken meat, *Appl. Microbiol.* 5:319-321, 1957.
5. WRENSHALL, C. L.: Advances in food technology made possible through the use of antibiotics. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc. 1957; p. 809.
6. TAYLOR, J. A., AND WALKER, E. A.: Recent developments in food preservation technology, *Research* 11:61-66, 1958.
7. YACOWITZ, H.; PANSY, F.; WIND, S.; STANDER, H.; SASSMAN, H. L.; PAGANO, J. F., AND TREJO, W. H.: Use of nystatin (Mycostatin) to retard yeast growth on chlortetracycline-treated chicken meat, *Poultry Sc.* 36:843-849, 1957.

# One Year's Experience of the Staphylococcus Reference Laboratory, Batavia, New York

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During 1956 and the first half of 1957, we experienced in the Batavia Veterans Administration Hospital a localized epidemic of staphylococcal infections. At that time considerable effort was made to study and characterize the various strains of bacteria incriminated in this epidemic. This small beginning under the impetus of the Chief Medical Director and the Chief of Research Service in the Veterans Administration was enlarged to a cooperative study involving six hospitals.

Using the six hospitals as representative institutions in the Veterans Administration, a survey of the extent of staphylococcal complications was made. Since prior information obtained from the Monthly Surgical Report did not give evidence that these kinds of infections were of significant importance, the study was set up to include both postsurgical and medical complications. An additional laboratory study included an evaluation of the sensitivity testing methods and an effort toward the standardization of these methods.

## MATERIALS AND METHODS

Six Veterans Administration hospitals (Butler, Pa.; Buffalo, N. Y.; Batavia, N. Y.; Syracuse, N. Y.; Manchester, N. H.; and Brooklyn, N. Y.) with a total bed capacity of 3326 were the original participating institutions. Since the study to be described was primarily of surgical origin, the first emphasis was focused on surgical wound complications. Of secondary interest, the medical infections and infections involving hospital personnel were considered. In all cases the pathology was considered to be significant if staphylococci were isolated from the infection or lesion. All cultural isolations made in the participating hospitals were forwarded along with a résumé of clinical data and laboratory determinations concerning the staphylococcal strains to the Reference Laboratory for further study. The laboratories of the participating hospitals performed the original antibiotic sensitivities so that there was no delay in placing the patient on an appropriate antibiotic. These antibiotic sensitivities were repeated in the Reference Laboratory and compared with the results of the initiating laboratory. The findings of the Reference Laboratory were compiled and returned to the initiating laboratory so that the results could be compared. The history chart of each infection was forwarded to the Chairman of the staphylococcal study group for statistical evaluation. After thorough trials of different antibiotic sensitivity testing materials, discs from one company were selected as giving the most reproducible results. These discs are also used in the participating laboratories. The sensitivity tests are controlled by parallel five tube dilution determinations. Initially about one in every five sensitivity determinations was controlled with the tube test. Later, the tube tests have of necessity been less frequent. The phage typing of all strains submitted was accomplished according to the method described by Blair.<sup>1</sup> Thirty-three bacteriophage are presently being employed for the phage typing. The Reference Laboratory has produced and maintained the phage filtrates for use in the typings. The coagulase determination of the staphylococci was determined initially by the

TABLE I  
*Antibiotic Spectrum*

Drug	Sensitive	Moderately resistant	Resistant	Per cent sensitive	Per cent moderately resistant	Per cent resistant	Per cent sensitive & moderately resistant
Penicillin	189*	98	576	22.0	12.0	66.0	34.0
	77	22	451	14.0	4.0	82.0	18.0
Tetracycline	309	31	523	36.0	3.0	61.0	39.0
	172	7	371	31.2	1.3	67.5	32.5
Chloramphenicol	578	172	113	67.0	20.0	13.0	87.0
	378	57	115	68.7	10.4	20.9	79.1
Erythromycin	523	64	276	61.0	7.0	32.0	68.0
	250	31	269	45.5	5.6	48.9	51.1
Novobiocin	705	120	4	85.0	14.5	0.5	99.5
	430	114	6	78.2	20.7	1.1	98.9
Streptomycin	217	172	454	26.0	20.0	54.0	46.0
	169	60	321	30.7	10.9	58.4	41.6
Neomycin	242	593	8	29.0	70.0	1.0	99.0
	400	150	0	71.7	27.3	0	100.0
Bacitracin	492	362	9	57.0	42.0	1.0	99.0
	244	301	5	44.4	54.7	0.9	99.1

\* Upper figures represent the first 863 specimens; lower figures represent the last 550 specimens.

test tube method but now is being determined with a capillary tube method. The pigment formation was determined by maintaining the organisms on Bacto *Staphylococcus* medium no. 110. The hemolytic reaction of the organisms was determined from the blood plates used for the sensitivity tests. The medium employed for these tests is a Tryptose agar base with 5 per cent human blood added.<sup>2</sup>

#### RESULTS

During the past year, 2087 specimens have been studied by the Reference Laboratory. The range of pathogenic processes has been from fatal staphylococcal pneumonia to minor skin infections and carriers. Although some patients have been studied more than once, it is now possible to state with some concrete basis that these infections are of considerable importance and that these complications do present an important problem. The exact infection rate in the over-all study is still to be determined. The one complicating factor in this kind of study is that the reported rate of infection varies with the enthusiasm and thoroughness of the on-the-spot investigator. Crude infection rate calculations run as high as 15 per cent of a hospital population at a given time.

A survey of the results of the antibiotic sensitivity testing has revealed that the disagreement between the initiating hospital and the Reference Laboratory may

range from 1 per cent with bacitracin and novobiocin to 43 per cent with chlorotetracycline; the average disagreement being 13.9 per cent. These figures were obtained during the early stages of this study when no specific instructions had been sent to the participating laboratories other than the fact that the sensitivity discs were furnished by the same company. A recent survey conducted with organisms of unknown sensitivity and specific instructions to the cooperating laboratories showed that less than 1 per cent disagreement was possible in sensitivity determinations. These instructions to the participating laboratories included the kind and quantity of medium to be used, the amount and age of inoculum, and the temperature and time of incubation. Table I shows the sensitivity results of the first 863 specimens and most recent 550 specimens. About one year separates the collection of the different data. Organisms received by the Reference Laboratory fall into three types: (1) The coagulase-positive phage typable group; (2) the coagulase-negative nontypable group, generally *Staphylococcus albus*; and (3) a coagulase-negative nontypable large coccus group. The latter organisms probably should be classified as *Gaffyka* or *Sarcina*. The results of the phage typing have disclosed that 19.3 per cent of the organisms studied were of the 42B, 52, 81 type. A relatively new type, 47, 77, 54, 53 has been appearing with greater frequency until 16.9 per cent of the strains studied are of this type. Of the specimens received, 24.4 per cent are nontypable. This includes 19.1 per cent of the coagulase negative, which were nontypable, and 5.3 per cent coagulase positive, which were nontypable. The correlation between the coagulase test, either the tube or capillary tube method, and phage typability has been 94.7 per cent. One reason for this high correlation was due to the fact that we have routinely used a phage testing dose, which is a 1 to 100 dilution of the undiluted lytic filtrate and in no case is the titer of the filtrates lower than 1 to 10,000.

#### DISCUSSION

The initial question that we set out to investigate has been answered. A significant number of postsurgical and medical infections have occurred in the hospitals under study. These infections have been of increased importance because of the additional time of hospitalization with sometimes serious consequences. In some institutions the problem of increased medical and nursing care has been acute, and, finally, poor control of these infections has resulted in considerably increased drug costs.

One of the most tangible results of the past year's study has been the formation of a more effective team to prevent and treat staphylococcal infections in each hospital. More reliable and faster laboratory determinations are now possible, with the result that an infection is recognized earlier and the patient placed on the appropriate antibiotic sooner. The separate laboratory problem of evaluation of sensitivity testing methods and the standardization of these methods was slow in getting started. This was due partially to the natural resistance to changed methods and partially to the unreliability of the existing sensitivity testing discs. In a few cases the inability to assign properly trained laboratory personnel to the bacteriology department was a problem. However, the latest survey of our six hospitals proves that a considerable agreement of standardization has resulted. More than 99 per cent agreement of sensitivity results was obtained. Increased laboratory interest in the staphylococcal problem has also increased the caliber of bacteriology work being performed.

The role of the reference laboratory in this kind of study was only partially defined when the work began. However, our experience has allowed the laboratory to grow into a varied range of functions. In most cases it is not possible for the usual hospital laboratory to assign an adequately trained bacteriologist to a full-time position of this nature. If detailed records are maintained, a considerable wealth of information can be secured by a laboratory of this kind. It has been possible to study thoroughly and standardize the drug sensitivity testing methods now in use.

#### SUMMARY

A study of 2087 staphylococcal isolations has been made. The changing state of bacterial sensitivities is emphasized. The high degree of correlation between virulence coagulation reaction and phage typability is reported and discussed. The functions of the reference laboratory are outlined and some of the benefits of such a function defined. The need for standardized procedures and sufficient trained personnel is stressed.

#### ACKNOWLEDGMENTS

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#### BIBLIOGRAPHY

1. BLAIR, J. E., AND CARR, M.: The bacteriophage typing of staphylococci, *J. Infect. Dis.* 93: 1-13, July-August, 1953.
2. GUTHRIE, F.: A study of methods for determining sensitivity of bacteria to antibiotics and the factors influencing differences in results, *Am. J. Med. Tech.* 24: 109-118, March-April, 1958.

# Occurrence, Phage-Type, and Antibiotic Susceptibility of Staphylococci in Various Community Groups

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The problem of antibiotic-resistant strains of *Staphylococcus aureus* has continued unabated since its status was summarized by Welch<sup>1</sup> in 1953. The problem concerns mainly those persons receiving antibiotics as therapeutic measures or those in close contact with antibiotics.

It was felt that useful information would be gained by studying the nasal flora of people from several community groups and determining the incidence of coagulase-positive staphylococci, the phage types of such cultures, and their antibiotic resistance patterns.

## EXPERIMENTAL

The following four community groups were studied for nasal carriage of coagulase-positive *Staph. aureus*:

1. A laboratory group consisted of 81 members of the Division of Antibiotics, a semi-closed group which, during the working day, handles and tests a great variety of antibiotic powders and solutions. Since the working area is air-conditioned (separated from the rest of the building and using about 75 per cent recirculated air) the personnel undoubtedly breathe a variety of antibiotic dusts and aerosols from the opening, weighing, diluting, and numerous manipulations involved in testing samples. For the same reason, both laboratory and clerical workers were included. In the case of this group it was possible to compare the results with those obtained with cultures isolated five years previously.

2. One hundred eight patients in a hospital-home for the aged made up a closed community, living in the same building and rarely leaving. A number of them had recently received or were currently receiving antibiotic therapy.

3. Forty members of the medical, nursing, and supporting staff of the hospital-home for the aged were a semi-closed community, in contact with each other and with the residents of the home only during their duty hours.

4. This consisted of a normal control group of 101 volunteer blood donors from all sections of the metropolitan area of Washington, D. C.

## METHODS

The anterior nares of each person were swabbed with sterile cotton swabs moistened with sterile Trypticase soy broth. Each swab was streaked thoroughly on the surface of sterile *Staphylococcus* medium 110 (Difco) in a Petri dish. The plates were incubated first for 24 hours at 32 C. and then 24 hours at 25 C., after which they were examined for colonies of pigment-producing *Staph. aureus*. The cultures thus obtained were isolated and maintained in pure culture on the same agar. They were tested for coagulase activity, and only those that were coagulase-positive were carried through the phage typing and antibiotic susceptibility tests; thus, any cultures

referred to in this paper are pigment-producing, coagulase-positive strains of *Staph. aureus*. The phage typing was done by the modified method<sup>2</sup> of Blair and Carr,<sup>3</sup> using 22 or, in some cases, 23 test phages.

The susceptibility of each culture of *Staph. aureus* was determined for the following antibiotics: chloramphenicol, erythromycin, novobiocin, oleandomycin, penicillin, streptomycin, and tetracycline. The twofold serial dilution tube technique was used, employing Trypticase soy broth. The inoculum was a 1:1000 dilution of an 18 to 24 hour culture of the organism in Trypticase soy broth. After overnight incubation at 37 C., the least amount of antibiotic resulting in complete inhibition of growth was recorded as the minimal inhibitory concentration.

## RESULTS

**Laboratory Group.** Of the 81 subjects, 17 (21 per cent) yielded cultures of *Staph. aureus*, 5 giving one culture each and 12, two cultures each. As shown in table I, of these 29 cultures, 17 (58.6 per cent) were of the phage group 0 (not susceptible to any of the test phages), 10 (34.5 per cent) were of groups III and I-III (a mixed type lysed by phages characteristic of both groups) and 2 (6.9 per cent) were of group IV. Nineteen cultures (65.5 per cent) were resistant to one or more antibiotics. Of the 203 susceptibility tests against seven antibiotics, 49 (24.2 per cent) indicated resistance (see footnote to table I). All but five of the cultures of phage group 0 were resistant to one or more antibiotics and accounted for 36 (73.5 per cent) of the tests indicating resistance. Of the 17 people yielding cultures, 12 (70.6 per cent) had one or two that were antibiotic resistant.

**Hospital Patients.** Cultures of *Staph. aureus* were obtained from 48 (44.5 per

TABLE I  
Twenty-Nine *Staph. aureus* Cultures from 81 People in an  
Antibiotics Testing Laboratory\*

No. cultures	Phage pattern	Phage group	Resistant† to
2	0	0	EOPST
2	0	0	EOST
1	0	0	EPS
4	0	0	PST
1	0	0	P
1	0	0	T
1	0	0	S
5	0	0	—
1	6/47/53/54/81	III	—
1	6/47/53/81	III	—
1	7/47/54/75/81	III	—
1	80	III	PST
1	81	III	ST
1	81	III	S
1	52/80	I-III	PST
1	52/80/81	I-III	—
1	52/52A/80	I-III	ST
1	52/77/52A/80	I-III	P
1	42D	IV	—
1	42D	IV	P

\* The 29 cultures were obtained from 19 persons; 12 yielded 2 cultures each and 7 yielded 1 culture each. The other 64 persons yielded no *Staph. aureus* cultures.

† Resistance was adjudged if the minimal inhibitory concentrations were found to be in excess of the following values: chloramphenicol (C), 20 µg./ml.; erythromycin (E), 5 µg./ml.; novobiocin (N), 5 µg./ml.; oleandomycin (O), 5 µg./ml.; penicillin (P), 5 u./ml.; streptomycin (S), 20 µg./ml.; and tetracycline hydrochloride (T), 20 µg./ml.

TABLE II

*Eighty-Four Staph. aureus Cultures from 108 Patients  
of Hospital-Home for Aged\**

No. cultures	Phage pattern	Phage group	Resistant <sup>†</sup> to
31	0	0	—
4	0	0	PST
1	79	I	—
3	7	III	—
1	6/47/53/54/81	III	—
1	6/47/81	III	—
1	6/81	III	T
2	54	III	PS
1	54	III	T
1	54/75/81	III	—
1	54/81	III	—
1	80/52AV	III	EPST
3	80/52AV	III	PST
1	81	III	T
5	81	III	—
2	52AV	III	PST
1	52AV	III	EPST
1	52AV	III	ST
1	29/80/81	I-III	—
12	52/80/81/52AV	I-III	EPST
1	52/80/81/52AV	I-III	—
4	52/80/81/52AV	I-III	PST
1	52/80/52AV	I-III	PST
1	3B/3C/55/6/81	II-III	—
1	3B/3C/55/81	II-III	—
1	3B/6/42E/81	II-III	—
1	55/6	II-III	T

\* The 84 cultures were obtained from 48 persons; 36 yielded 2 cultures each and 12 yielded 1 culture each. The other 60 persons yielded no *Staph. aureus* cultures.

<sup>†</sup> See table I<sup>†</sup> for resistance criteria.

cent) of the 108 hospital patients, with 12 yielding one culture each and 36 yielding two cultures each. As shown in table II, of these 84 cultures, 35 (41.6 per cent) were of phage group 0, 1 (1.2 per cent) of group I, 44 (52.4 per cent) of groups III and I-III, and 4 (4.8 per cent) of the mixed group II-III. Thirty-five cultures (41.7 per cent) were resistant to one or more antibiotics. Of the 588 antibiotic susceptibility tests, 108 (18.4 per cent) indicated resistance. Only 4 of the 35 phage group 0 cultures were antibiotic-resistant. Within phage groups III and I-III, of the 26 cultures susceptible to phage 52AV, 25 were resistant to one or more antibiotics and accounted for 88 (74.5 per cent) of the 108 tests indicating resistance. Of the 48 patients yielding cultures, 20 (41.7 per cent) had either one or two that were antibiotic resistant.

*Hospital Personnel.* Of the 40 persons tested, 17 (42.5 per cent) yielded cultures of *Staph. aureus*, with 3 giving one culture each and 14 two cultures each. Table III shows that of these 31 cultures, 10 (32.2 per cent) were of phage group 0, 2 (6.5 per cent) of group I, 2 (6.5 per cent) of group II, and 17 (54.8 per cent) of groups III and I-III. Thirteen cultures (42 per cent) were resistant to one or more antibiotics. Of the 217 antibiotic susceptibility tests, 41, (18.9 per cent) indicated resistance. Of the 10 phage group 0 cultures, only 1 was antibiotic-resistant. Within groups III and I-III, of the 10 cultures susceptible to phage 52AV, all were resistant to one or more antibiotics and accounted for 34 (82.8 per cent) of the 41 tests indicating resistance. Of the 17 people yielding cultures, 11 (64.8 per cent) had either one or two that were antibiotic-resistant.

*Control Group.* Of the 101 people used as normal controls, 48 (47.5 per cent) yielded cultures of *Staph. aureus*, with 19 yielding one culture each, and 29 two cultures each. Table IV shows that of the 77 cultures, 48 (62.3 per cent) were of phage group 0, 8 (10.4 per cent) of group II, 18 (23.4 per cent) of groups III and I-III, and 2 of mixed group I-III-IV. Five cultures (6.5 per cent) were antibiotic-resistant, *but to only one antibiotic each*. Four were of group 0 and resistant to tetracycline; one was of group III and resistant to penicillin. Of the 539 susceptibility tests then, only 5 (0.9 per cent) indicated resistance. Of the 48 people yielding cultures, only 4 (8.3 per cent) had either one or two that were antibiotic resistant.

*Comparison of Results.* In 1953, 69 of the personnel of the Division of Antibiotics were tested. *Staph. aureus* was cultured in 8 (11.6 per cent), as compared to the 21 per cent in the 1958 survey. However, in 1953 only one nostril was swabbed, while both nares were tested in 1958. Thirty-eight of the same individuals were tested both in 1953 and in 1958. Of these 38, 7 (18.4 per cent) yielded cultures of *Staph. aureus* in 1953, and 9 (23.6 per cent) yielded cultures in 1958. Twenty-five were negative both times. Of the 7 positive in 1953, only 3 were positive in 1958. Six who were negative in 1953 were positive in 1958. None of the phage patterns found in 1953 were duplicated in 1958, except for those of phage 0 (not susceptible to any of the test phages). While the 1953 cultures were not tested for susceptibility to novobiocin, oleandomycin, or tetracycline, none was adjudged resistant to chloramphenicol or erythromycin at that time. Three of the 7 1953 cultures were resistant to penicillin, and 4 were resistant to both penicillin and streptomycin. The 9 individuals who were positive in 1958 yielded 15 cultures, of which 7 were susceptible to all seven test antibiotics; 1 was resistant to penicillin; 1 to streptomycin; 2 to streptomycin and tetracycline; 2 to penicillin, streptomycin, and tetracycline; and 2 to erythromycin, oleandomycin, penicillin, streptomycin, and tetracycline.

TABLE III  
*Thirty-One Staph. aureus Cultures from 40 Persons  
on the Staff of Hospital-Home for Aged\**

No. cultures	Phage pattern	Phage group	Resistant† to
9	0	0	—
1	0	0	PT
1	79	I	—
1	79	I	S
1	3B/3C	II	EPST
1	3B/3C	II	—
1	7	III	—
1	42E/81	III	—
1	6/42E/75/81	III	—
2	81	III	—
1	80/52AV	III	PT
1	52/80/81/52AV	I-III	EPT
5	52/80/81/52AV	I-III	EPST
2	52/80/81/52AV	I-III	PST
1	52/80/81/52AV	I-III	EPS
1	52/81	I-III	—
1	79/81	I-III	—

\* The 31 cultures were obtained from 17 persons; 14 yielded 2 cultures each and 3 yielded 1 culture each. The other 23 persons yielded no *Staph. aureus* cultures.

† See table I† for resistance criteria.

TABLE IV  
*Seventy-Seven Staph. aureus Cultures from a Control Group of  
101 Normal Subjects (Blood Donors)\**

No. cultures	Phage pattern	Phage group	Resistant† to
44	0	0	—
4	0	0	T
4	3A	II	—
4	3A/3C	II	—
2	6/42E/47/81	III	—
1	6/47/77	III	P
2	7/42E/47/54/75/81	III	—
1	7/47/54/75/81	III	—
1	55	III	—
1	70/81	III	—
1	81	III	—
2	52/6/42E/47/53/81	I-III	—
1	52/47/53	I-III	—
1	52/47/53/54/81	I-III	—
2	52/80/81	I-III	—
2	52/52A/80/81	I-III	—
2	52/81	I-III	—
2	44A/81/42D	I-III-IV	—

\* The 77 cultures were obtained from 48 persons; 29 yielded 2 cultures each and 19 yielded 1 culture each. The other 53 persons yielded no *Staph. aureus* cultures.

† See table I† for resistance criteria.

#### DISCUSSION

A number of significant group-to-group relationships are indicated in table V. While the carrier rates of the control group and the two hospital groups were within the rather narrow range of 42.5 to 47.5 per cent, the laboratory carrier rate was only 21 per cent. This disparity may be due to an over-all depression of the staphylococcal population in the nares by an overwhelming concentration of antibiotics in the environment. This laboratory handles what is probably the widest variety of antibiotics possible, including not only every one of those commercially available but also a number of experimental ones. Moreover, those cultures that do survive in the laboratory personnel appear to be more resistant to antibiotics than those of the other groups. Of the cultures from the laboratory group, 65.5 per

TABLE V  
*Staphylococcus aureus Cultures from 330 Persons in Four Community Groups*

Observations	Laboratory personnel	Hospital patients	Hospital personnel	Control group
Individuals tested	81	108	40	101
No. with 1 culture	5	12	3	19
No. with 2 cultures	12	36	14	29
Total with cultures	17	48	17	48
Carrier rate (per cent)	21	44.5	42.5	47.5
No. of carriers with resistant cultures	12	20	11	4
Per cent of carriers with resistant cultures	70.6	41.7	64.8	8.3
Swabs tested	162	216	80	202
Cultures found	29	84	31	77
Resistant cultures	19	35	13	5
Per cent resistant	65.5	41.7	42	6.5
Susceptibility tests (different antibiotics)	203	588	217	539
No. tests indicating resistance	49	108	41	5
Per cent of tests indicating resistance	24.2	18.4	18.9	0.9

cent were resistant to one or more antibiotics, while 42 per cent of those from the hospital groups and only 6.5 per cent of those from the control group were antibiotic resistant.

The unique nature of the cultures from the laboratory group is also reflected in the large percentage of susceptibility tests indicating resistance to the seven test antibiotics. Antibiotic resistance was indicated in 24.2 per cent of the tests on cultures from the laboratory group, 18.4 per cent of those from the hospital patients, 18.9 per cent of those from the hospital staff, and only 0.9 per cent of those from the control group. However, multiple resistance appeared to be a little more common in the hospital groups than in the laboratory group. Multiple antibiotic resistance occurred in 88.6 per cent of the resistant cultures from the hospital patients, 92.3 per cent of those from the hospital staff, and only 68.4 per cent of those from the laboratory group. Multiple resistance was not found in the control group.

The carrier rates of 42.5 to 47.5 per cent in the control group and the two hos-

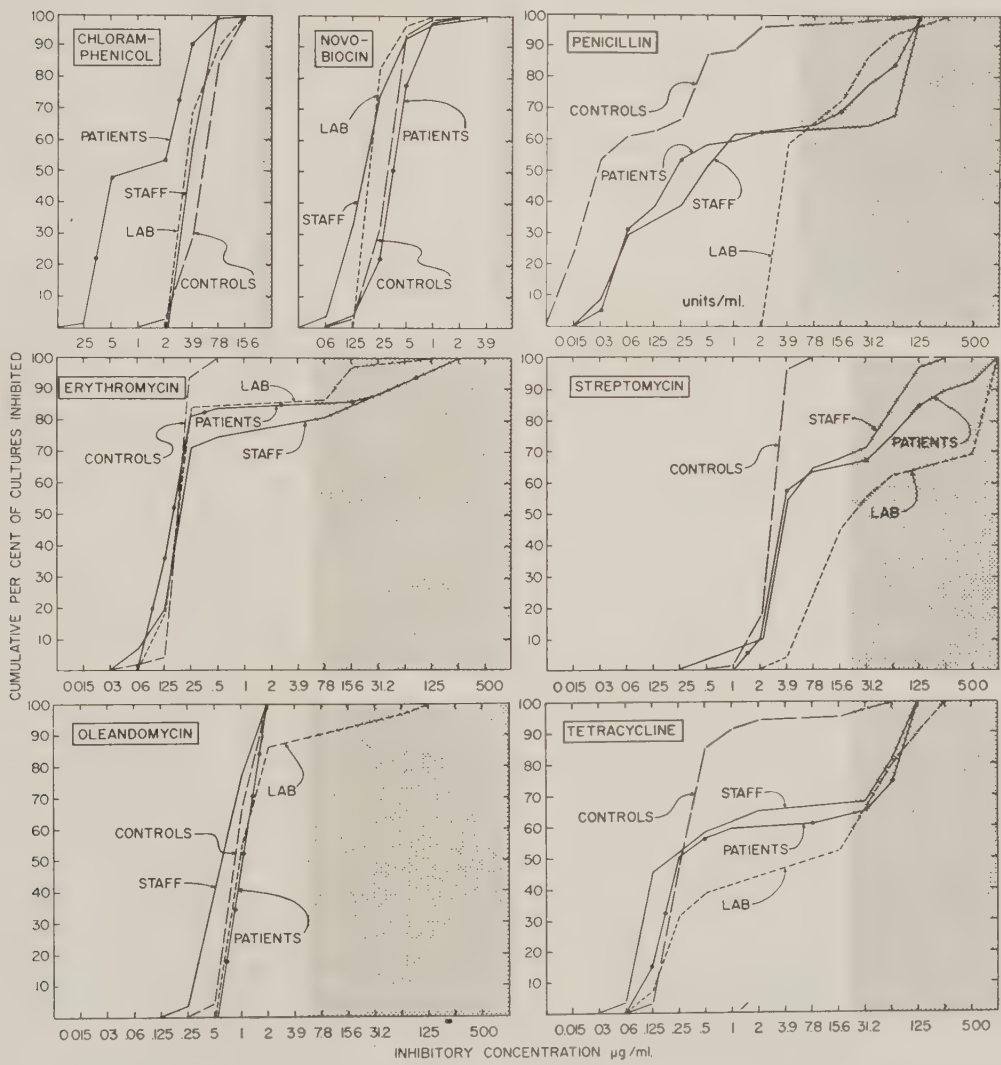


FIG. 1. Cumulative per cent of *Staphylococcus aureus* cultures from four community groups susceptible to various antibiotics. Number of cultures from each group: Laboratory group (LAB), 29; hospital patients (PATIENTS), 84; hospital staff (STAFF), 31; and control group (CONTROLS), 77. Areas of antibiotic resistance are shaded.

pital groups agree well with the rates observed by a number of workers. Recently, Caswell et al<sup>4</sup> reported the carrier rate to be 41 per cent among 640 hospital personnel, and Gould<sup>5</sup> reported it to range from 27 to 32 per cent among 96 people from a shipping department, an office, and the general population. Moreover, Gould<sup>5</sup> also found the carrier rate to be only 9 per cent among a group of workers in a penicillin factory. This lower carrier rate may be compared with the low 21 per cent obtained in our laboratory group.

Figure 1 shows the susceptibility of the cultures from each of the four community groups to the seven test antibiotics. There was relatively little difference in the susceptibility of all organisms to chloramphenicol or novobiocin, although many of the cultures of the hospital patients appeared to be more susceptible to chloramphenicol than those of the other groups. In the case of erythromycin, the cultures from the control group were extremely susceptible, while approximately 20 per cent of the cultures from the other three groups were resistant. All cultures were quite susceptible to oleandomycin, except about 13 per cent of those from the laboratory group which were resistant.

The most noticeable differences were in the susceptibilities of the four groups of cultures to penicillin, streptomycin, and tetracycline. The cultures from the control group were significantly more susceptible to these three antibiotics. The cultures from the hospital patients and the hospital staff were remarkably similar in their susceptibility and resistance. The cultures from the laboratory group were consistently more resistant than the other cultures. Although about 60 per cent of the cultures from the hospital groups and the laboratory group were susceptible to penicillin, those from the laboratory group required considerably more of the antibiotic for inhibition than did the others.

Cultures from the laboratory group were more resistant than the others to streptomycin and tetracycline.

These differences may be related to the extent to which the various groups are exposed to the given antibiotics. Of course, the control group is made up of essentially normal and healthy people having a minimum of contact with all kinds of antibiotics. Hence, their nasal organisms are principally antibiotic-susceptible. The relationship of resistance of nasal cultures to environmental factors or extent of use apparently does not apply to chloramphenicol and novobiocin, since all cultures were susceptible to them to about the same degree. With oleandomycin, the resistance of 13 per cent of the laboratory cultures may reflect the increased testing of new products containing this antibiotic.

The similarity of the cultures from the two hospital groups may be due to the close contact between them in the environment of the same building day after day.

A summary of the susceptibility of all of the 221 cultures from all groups follows. It may be seen that the criteria established for judging the cultures to be susceptible or resistant were such that, had they been set at either one-half or twice that inhibitory concentration, there would have been little difference in the calculated rates of resistance.

*Chloramphenicol.* Although 20  $\mu\text{g./ml.}$  was established as the borderline for resistance, it is obvious from figure 1 that all cultures were susceptible to 15.6  $\mu\text{g./ml.}$  or less, and 92.8 per cent were susceptible to 7.8  $\mu\text{g./ml.}$  or less.

*Erythromycin.* The borderline for resistance was set at 5  $\mu\text{g./ml.}$  Of the 27 cultures (12.2 per cent) classified as resistant, all but 1 required 15.6  $\mu\text{g./ml.}$  or more for inhibition. All of the 194 erythromycin-susceptible cultures were susceptible to 0.5  $\mu\text{g./ml.}$  or less.

*Novobiocin*. Although 5  $\mu\text{g./ml.}$  was selected as the borderline for resistance, all cultures were susceptible, 1 to 3.9  $\mu\text{g./ml.}$ , 3 to 2  $\mu\text{g./ml.}$ , 23 to 1.0  $\mu\text{g./ml.}$ , and 194 were susceptible to 0.5  $\mu\text{g./ml.}$  or less.

*Oleandomycin*. The borderline for resistance was set at 5  $\mu\text{g./ml.}$  The 4 resistant cultures (1.8 per cent) required 62.5  $\mu\text{g./ml.}$  or more for inhibition, while of the remaining 217, 78 were inhibited by 2  $\mu\text{g./ml.}$ , 135 by 1.0  $\mu\text{g./ml.}$ , and 4 by 0.5  $\mu\text{g./ml.}$  or less.

*Penicillin*. The critical value was set at 5 u./ml. Of the 57 cultures (25.8 per cent) classified as resistant, 1 required 7.8 u./ml. for inhibition, 8 required 15.6 u./ml., and 48 required 31.2 u./ml. or more. Of the 164 susceptible cultures, 18 were inhibited by 3.9 u./ml., 9 by 2 u./ml., and 137 by 1.0 u./ml. or less.

*Streptomycin*. Cultures requiring greater than 20  $\mu\text{g./ml.}$  for inhibition were adjudged resistant. Of 57 (25.8 per cent) resistant cultures, seven required 31.2  $\mu\text{g./ml.}$  and 50 required 62.5  $\mu\text{g./ml.}$  or more. Of the 167 susceptible cultures, 13 were inhibited by 15.6  $\mu\text{g./ml.}$ , 11 by 7.8  $\mu\text{g./ml.}$ , and 143 by 3.9  $\mu\text{g./ml.}$  or less.

*Tetracycline*. Cultures inhibited by greater than 20  $\mu\text{g./ml.}$  were considered resistant. Of the 62 (28 per cent) resistant cultures, 10 required 31.2  $\mu\text{g./ml.}$  for inhibition, and 52 required 62.5  $\mu\text{g./ml.}$  or more. Of the 159 susceptible cultures, 6 were inhibited by 15.6  $\mu\text{g./ml.}$ , 1 by 7.8  $\mu\text{g./ml.}$ , 3 by 2  $\mu\text{g./ml.}$ , and 149 by 1.0  $\mu\text{g./ml.}$  or less.

*Phage Patterns*. It is of interest to compare the phage patterns observed in the four community groups. Those not susceptible to any of the test phages (group 0) comprised 58.6 per cent of the cultures from the laboratory group, 41.6 per cent of those from the hospital patients, 32.3 per cent of those from the hospital personnel, and 62.4 per cent of those from the control group. However, while resistance was indicated in 30.2 per cent of the susceptibility tests carried out on the group 0 cultures from the laboratory group, this held true for only 4.9 per cent of the tests on group 0 cultures from the hospital patients, 2.9 per cent of the hospital staff, and as little as 1.2 per cent of the control group.

The predominant phage groups in the hospital patients and personnel were those of group III and the mixed group I-III; these comprised 52.4 per cent of the cultures from the patients and 51.7 per cent of the cultures from the hospital staff. Phage groups III and I-III comprised 34.4 per cent of the cultures from the laboratory group and only 23.4 per cent of those from the control group. Of the susceptibility tests performed, 30.8 and 30.4 per cent, respectively, of those in the group III and I-III cultures from the hospital patients and personnel indicated antibiotic resistance, while 17.1 per cent of the laboratory group, and only 0.8 per cent of the control group did so. Phage groups I, II, and IV were encountered only rarely, except in the control group, which yielded seven cultures (9 per cent) of group II. Four cultures of the mixed type II-III were encountered in this group of patients.

It is interesting to note that the phage patterns found in the cultures of the laboratory group in 1953 did not include mixed group I-III to any extent. However, Jackson and co-workers, in 1954, found that between 10 and 20 per cent of their hospital isolates were of this mixed group. They reported that "a large proportion of the strains belonging to the mixed phage group I-III were not inhibited by high concentrations of chlortetracycline."<sup>6</sup>

The majority of reports published at that time, however, paralleled our 1953 findings, in that most of the organisms with either single or multiple antibiotic resistance were of phage group III. At the present time the picture has changed,

with organisms in the mixed phage group I-III being implicated frequently as antibiotic-resistant etiological agents in epidemics of *Staphylococcus* infections.<sup>4,8,9</sup>

The question naturally arises as to whether the mixed group I-III had its origin in group I types that have become susceptible to group III phages or vice versa. Regardless of the answer, it is apparent that cultures with any of the phage group III characteristics are predisposed to develop antibiotic resistance. However, as noted in the laboratory group, there is a relatively large number of untypable cultures resistant to phages and resistant to the antibiotics as well. This makes it difficult to delineate an exact relationship between phage type and antibiotic resistance, except within certain community groups.

*Staphylococcus* phage 52AV, proposed by Comtois and Bynoe<sup>7</sup> as a substitute for phages 80 and 81, was used in typing the cultures from the hospital groups but not the others. In the present study, there were 20 cultures from the hospital groups susceptible to phage 81 but not to 80 or 52AV; 1 susceptible to both 80 and 81 but not to 52AV; 6 susceptible to 80 and 52AV but not to 81; 4 susceptible to 52AV but not to 80 and 81; and 26 susceptible to all three, 80, 81, and 52AV. Of the 36 cultures susceptible to phage 52AV, 35 were resistant to one or more antibiotics (resistance was indicated in 122 of the 252 tests). Of the 21 susceptible to phages 80 and/or 81 but not 52AV, only 2 were resistant and then only to one antibiotic. This is taken as an indication that phage 52AV might be extremely useful in identifying staphylococcal cultures of potential hazard.

#### SUMMARY AND CONCLUSIONS

A study of nasal carriers of pigment-producing, coagulase-positive *Staphylococcus aureus* has been carried out among four community groups: (1) a laboratory group of 81 persons in a semi-closed situation engaged in testing a wide variety of antibiotics; (2) a closed group of 108 patients in a hospital-home for the aged; (3) a semi-closed group of 40 persons working in the hospital-home; and (4) a control group of 101 persons from the general population.

The cultures of *Staph. aureus* were phage typed and tested for susceptibility to chloramphenicol, erythromycin, novobiocin, oleandomycin, penicillin, streptomycin, and tetracycline.

The laboratory group had the lowest carrier rate (21 per cent) and the largest percentage of tests showing resistance (24.2 per cent).

The two hospital groups were similar to each other in that the carrier rates were 44.5 per cent for the patients and 42.5 per cent for the hospital staff, and the percentages of tests showing resistance were 18.4 and 18.9 per cent, respectively.

The control group had a carrier rate of 47.5 per cent, but only 0.9 per cent of the tests indicated antibiotic resistance.

In the laboratory group, 73.5 per cent of the tests indicating resistance were with cultures of phage group 0. In the two hospital groups, most of the antibiotic-resistant cultures were of phage group III or the mixed group I-III. Cultures susceptible to phage 52AV appeared to be particularly associated with antibiotic resistance, since they were involved in 74.5 per cent of the tests on patients' cultures showing antibiotic resistance and 82.8 per cent of the tests on the hospital staff's cultures showing resistance. Therefore, phage 52AV might be useful in identifying staphylococci of potential hazard.

Comparisons of the susceptibility of organisms from the four communities to each antibiotic individually showed all cultures to be susceptible to chloramphenicol

and novobiocin. All of the cultures from the control group were susceptible to erythromycin, oleandomycin, and streptomycin; 99 and 95 per cent were susceptible to penicillin and tetracycline, respectively. There was some resistance to erythromycin in the other three groups, ranging from 17 to 29 per cent. Oleandomycin resistance was encountered only in the laboratory group (13 per cent).

The hospital groups had very similar resistance patterns for penicillin (37 and 39 per cent resistant), streptomycin (37 and 33 per cent resistant), and tetracycline (39 and 35 per cent resistant). Forty-one per cent of the cultures from the laboratory group were classified as penicillin-resistant, but the penicillin-susceptible cultures appeared to be much less susceptible than cultures from the other groups. There were fewer streptomycin-susceptible organisms among the laboratory group, and more streptomycin-resistant ones (55 per cent) than in any other group. There were also fewer tetracycline-susceptible cultures among the laboratory group and more tetracycline-resistant ones (49 per cent).

These data indicate that the incidence of antibiotic resistance in nasal cultures of *Staph. aureus* is very closely related to contact with antibiotics and, possibly, with persons who are carriers of antibiotic-resistant strains. No one phage group could be implicated as the major one in the four groups studied, but, within circumscribed environments, certain types have been identified as predominantly antibiotic-resistant.

#### ACKNOWLEDGMENTS

The authors wish to express their gratitude to the Samuel Gompers Lodge 45, where the control group of blood donors was swabbed, and to the District of Columbia Chapter of the American Red Cross for allowing us to disrupt their usual routine. We are also grateful to the patients and staff of the Hebrew Home for the Aged for their cooperation, particularly to Mrs. Mae L. Segal, who greatly facilitated the isolation procedures with her efficient organization and direction. Phage 52AV was obtained from Dr. R. D. Comtois of the Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Canada.

#### BIBLIOGRAPHY

1. WELCH, H.: Editorial. The antibiotic-resistant staphylococci, *Antib. & Chemo.* 3:561-570, June, 1953.
2. OSWALD, E. J., AND REEDY, R. J.: A technic for the bacteriophage typing of staphylococci, *Antib. & Chemo.* 4:574, May, 1954.
3. BLAIR, J. E., AND CARR, M.: The bacteriophage typing of staphylococci, *J. Infect. Dis.* 93:1, 1953.
4. CASWELL, H. T.; SCHRECK, K. M.; BURNETT, W. E.; CARRINGTON, E. R.; LEARNER, N.; STEEL, H. H.; TYSON, R. R., AND WRIGHT, W. C.: Bacteriologic and clinical experiences and the methods of control of hospital infections due to antibiotic resistant staphylococci, *Surg., Gynec. & Obst.* 106:1-10, Jan., 1958.
5. GOULD, J. C.: Environmental penicillin and penicillin-resistant *Staphylococcus aureus*, *Lancet* 1:489-493, March 8, 1958.
6. JACKSON, G. G.; LEPPER, M. H., AND DOWLING, H. F.: Bacteriophage typing of staphylococci. III. Relationship of antibiotic sensitivity and resistance, *J. Lab. & Clin. Med.* 44:41-50, July, 1954.
7. COMTOIS, R. D., AND BYNOE, E. T.: Lytic reactions of three closely related staphylococcal bacteriophages 80, 81 and '52AV'. Proceedings of the 58th General Meeting of the Society of American Bacteriologists, April 27 to May 1, 1958, p. 139.
8. PETERSDORF, R. G.; CURTIN, J. A., AND BENNETT, I. L.: The sensitivity of two hundred strains of hemolytic *Staphylococcus* to a series of antibiotics, *A.M.A. Arch. Int. Med.* 100:927-936, 1957.
9. FEKETY, F. R.; BUCHBINDER, L.; SHAFFER, E. L.; GOLDBERG, S.; PRICE, H. P., AND PYLE, L. A.: Control of an outbreak of staphylococcal infections among mothers and infants in a suburban hospital, *Am. J. Pub. Health* 48:298-310, March, 1958.

# The Spread of Staphylococci to the Environment

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The patient is I. C., a 58 year old telegrapher with a 10 year history of infections. In 1948 she had repeated sore throats and swollen nodes in her neck. Following removal of her tonsils and adenoids, the swollen nodes disappeared. In 1954, this patient had boils in her armpits. Her physician cultured the pus and treated her with penicillin. It was dramatically effective, but ultimately the boils recurred. He told her the bacteria had become resistant to penicillin. After an interval, the boils stopped forming.

In 1955, the patient's husband became an invalid because of multiple myeloma. She nursed him at home until a boil on his left ankle and spread of this septic process caused hospitalization; death followed several months later. Throughout this period, the patient had crops of pimples. In 1956, she had boils again in her armpits. Several months later she had an outbreak of boils about the waistline, followed by one below the left breast. In October, 1957, she had the "grippe." Another physician gave her a single injection of penicillin, which was followed by high fever and a generalized rash. She was given prednisone, and, although the rash was somewhat improved, it persisted until admission.

Three weeks prior to admission, a small carbuncle developed on her left forearm. One week later, a similar lesion occurred in the small of her back. The following week, a larger carbuncle developed at the margin of the areola of her right breast. Hospitalization was advised to discover the cause for continued skin infections.

This patient illustrates two serious medical problems. One, the host-parasite relationship, is difficult to understand. The other, her relationship to environmental sepsis, is less obscure, because it can be studied readily.

Prior to the patient's admission, her hospital room was disinfected. The walls were sprayed with a germicidal detergent and dried. The furniture was washed with germicide. The floor was flooded with a detergent germicide, and the residual was picked up with a vacuum cleaner. At the end of the process, the floor, bedding, and air were cultured to determine the bacteriology of the environment. Periodically during her hospital stay, the bedding, the floor, and the air and room dust were cultured.

Cultures were also made of the patient's nasopharynx, gastric juice, anal tract, urine, the skin of the hands and feet, and each of the carbuncles. The *Staphylococcus* recovered from all sources, except the gastric juice, which was sterile, was of particular interest in the investigation of environmental sepsis, because it was atypical and strange to the hospital. Cultural characteristics were distinctive; colonies were minute; pigmentation occurred late; mannitol did not ferment; and plasma coagulated. The bacteria were sensitive to all antibiotics and of phage type group I, type 79.

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The bacteriological studies illustrate two important concepts too little appreciated by physicians: (1) the carbuncles were local manifestations of systemic staphylococcal disease; and (2) despite dry, sterile dressings, staphylococci were shed to her environment.

Because the staphylococci were atypical of those in the environment, they could be followed as they spread. Cultures obtained from the sheet and pillow cases when the bed was clean and after it had been used for 14 hours are shown in figure 1. Note the small colonies with limited pigmentation that grew where the used sheet and pillow case contacted the blood agar. The Well's air centrifuge tubes shown on the right in figure 2 were exposed to the air in the unoccupied room; those on the left to the air after the patient had used the room overnight.

Cultures made from dust picked up from the window sill in this patient's room are shown in figure 3. Note the staphylococcal colonies scattered throughout. The source of these organisms was proved to be this patient.

Figure 4 illustrates the spread of bacteria to the patient's room. Bacteria are whisked into the air in sufficient numbers by activity, such as bedmaking, to inoculate the nasopharynx of anyone who enters the area. The accumulation of bacteria on successive days makes the environment increasingly hazardous.

The *Staphylococcus* could be followed to the hospital laundry. Soiled laundry serves as a multiplier for bacteria. This is particularly true when the linens are wet with drool, perspiration, or from incontinence. As this laundry is processed, the bacteria contaminate personnel and are thrown into the air, where they become a source of contamination. Proper management of the laundry can eliminate this multiplier and feedback.

Cleansing the floor of the patient's room with a mop or broom contaminates these tools. Bacteria are thus carried to other patient areas and, in the case of the mop, to the mop pail. In the latter they multiply, and the next time the mop is

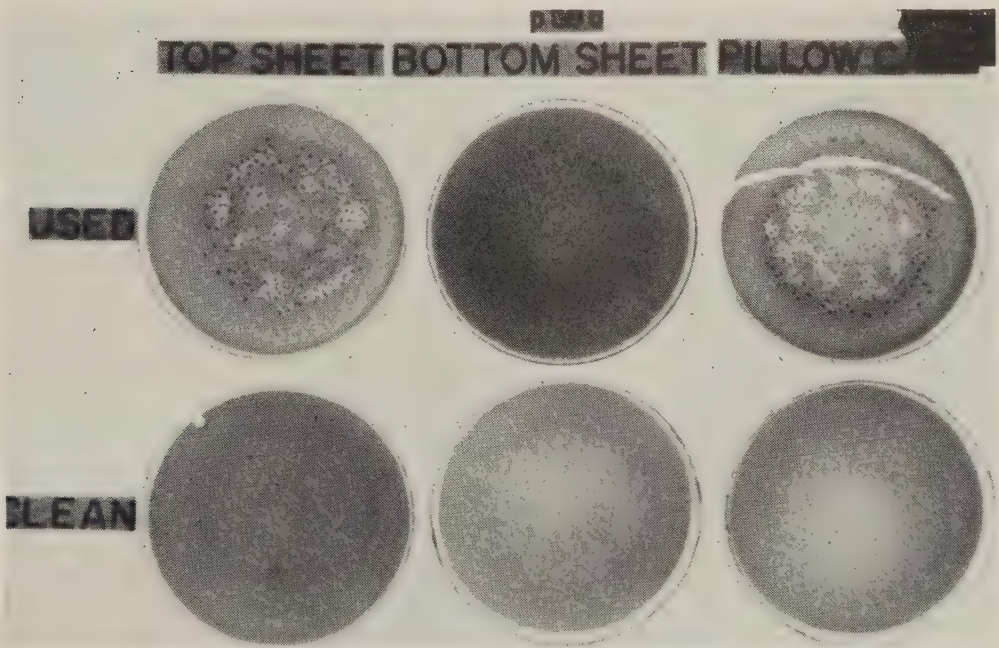


FIG. 1. Cultures obtained by pressing bedding against blood agar. (Lower) Before occupancy; (Upper) after 14 hours' occupancy.

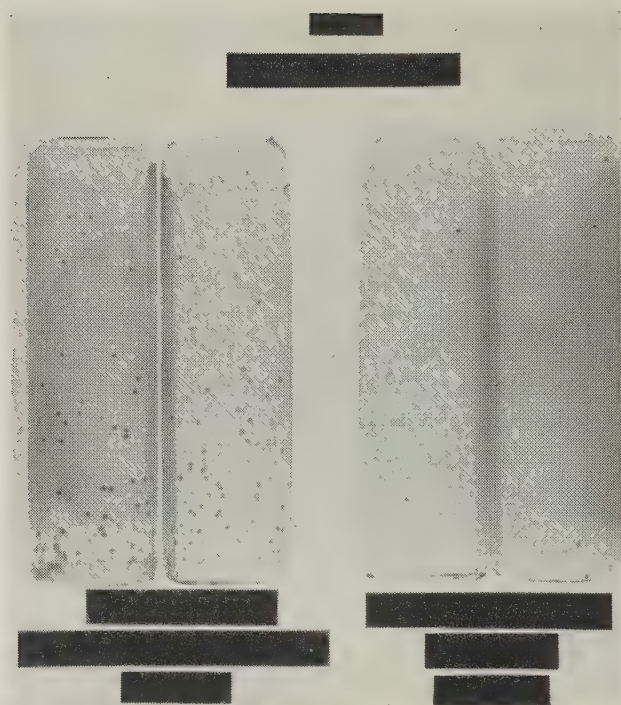


FIG. 2. Well's air centrifuge tubes inoculated prior to admission (*Left*) and during bedmaking on the morning following admission (*Right*).

used bacteria are painted on the floor. The floor count following this may rise from 50 to 3000/sq. cm.

Figure 5 illustrates the spread of sepsis from the environment to the portal of entry. Usually, the portal of entry is thought to be a freshly made wound. However, I am convinced that the chief portal of entry is a breach in the mucous membrane of the respiratory passages and that wound sepsis is often an expression of bacteremia, secondary to pulmonary vein thrombophlebitis, that follows septic embolization from traumatized mucous membrane in the nasopharynx. This trauma may be the result of intubation or chemical irritation. Inflammation resulting from intercurrent upper respiratory disease also opens this portal of entry to invasion by chance organisms picked up during the normal course of breathing, which involves eight liters of air per minute.

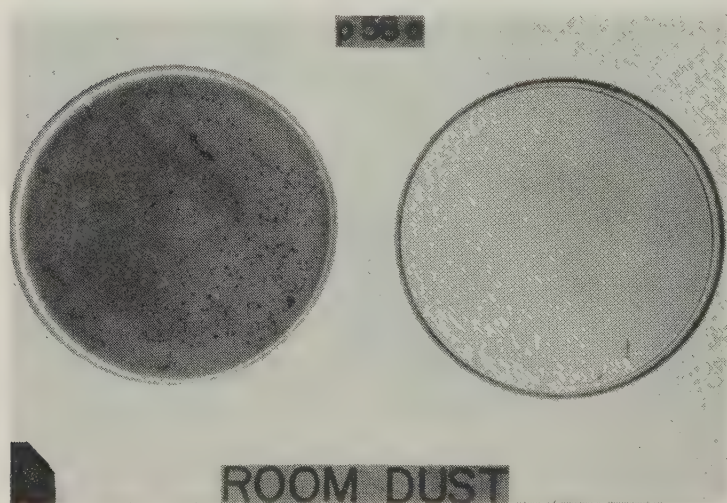
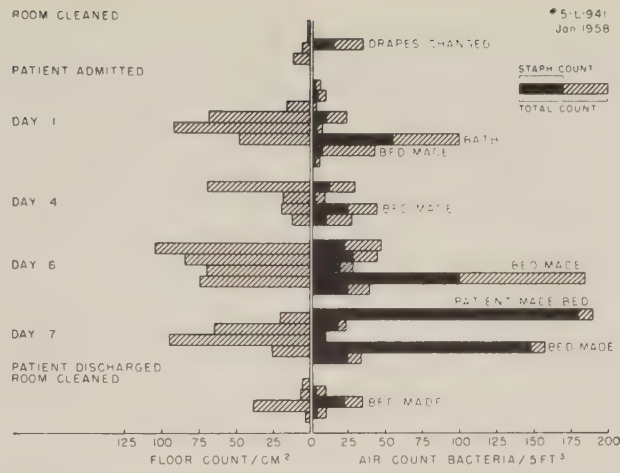


FIG. 3. Cultures made by passing swab over the window sill; (*Left*) after 14 hours' occupancy; (*Right*) before occupancy.

FIG. 4. Bacterial counts of floor and room air while patient was being cared for under isolation technique. Staphylococci recovered from air after drapes were changed were untypable; the remainder were type 79. This type persisted in the room following routine cleaning on discharge.

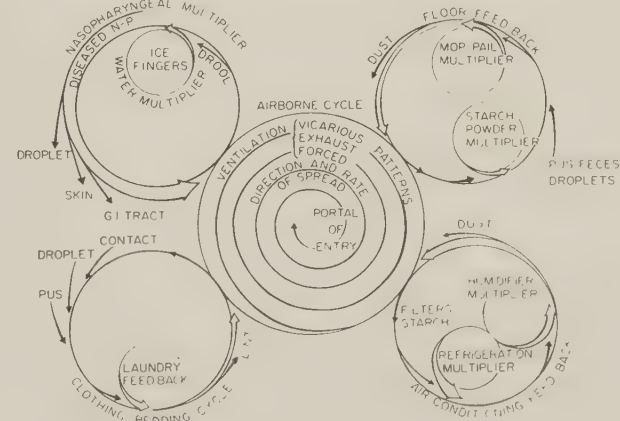


Air carries bacteria to the nasopharynx at a rate and from sources that can be determined by studying the ventilation patterns and practices in a hospital. Open fire doors, exhaust fans, and interruption of normal positive pressure ventilation invite relocation of bacteria from septic areas. Sterile air introduced into a clinical area is just as good a vehicle for carrying bacteria from a reservoir or multiplier as is unsterile air. Air becomes contaminated by bacteria mechanically freed from four chief multipliers: the nasopharynx of personnel and patients, the clothing and bedding, the air-conditioning, and the floor.

Dry dressings are customarily thought to be sterile. This is not so. Bacteria can be picked up by pressing a blood agar plate against the outer layer of a dry dressing. Wound discharges, carried into the dressing material by capillarity, dry and deposit bacteria in the outer layer of the dressing. Patients often contaminate their fingers on the dressings, or, more probable, wound bacteria establish themselves on the skin. This can be demonstrated in fingerprints on a culture plate. The septic patient, then, is the source of the bacteria. He must be strictly isolated. This is difficult to achieve in routine hospital practice. The ambulatory convalescent patient is an extreme hazard, unless he is bacteriologically safe.

Control must also be instituted to break the cycles of the ventilation vortex. Positive pressure ventilation must be instituted in all clinical areas. Open doors to stair wells and exhaust fans must be eliminated. Chutes and elevator shafts must be ventilated to prevent pumping of air between floors.

FIG. 5. Air-borne spread of bacteria by ventilation patterns of institution. Air transports bacteria from environmental multipliers and reservoirs to the nasopharynx and portal of entry.



The nasopharyngeal multiplier is readily identified by culture. The carrier is not a significant vector, as long as he is asymptomatic. He becomes a hazardous spreader of bacteria when the mucous membranes are inflamed by upper respiratory infections. At this time, the number of bacteria expelled from his nasopharynx increases two to three thousand per cent. They settle on his clothing and bedding; his skin becomes heavily contaminated. The air about a spreader is full of bacteria. Culture of his hair, clothing, or pillow differentiates the spreader from the benign carrier.

In many hospitals, there is another multiplier that must be considered—the bedside water carafe. This is often contaminated either by drool at the end of the sip through a straw or by bacteria from the fingers of those who process the ice and water. Table I shows densities of coliform bacteria and staphylococci cultured from bedside water carafes far exceed safe levels in 24 Boston hospitals. This is one of the multipliers that can be eliminated by daily heat sterilization of carafes and proper handling of ice.<sup>1</sup>

Bacteria discharged from the nasopharynx gradually settle out to the floor, where they encounter nutritive dust that supports multiplication. In the operating room there is starch powder. In clinical situations there is desquamating epithelium, dried saliva, feces, and other dusts which, when moistened by cleaning water, support bacterial growth.

The mop and pail have been described as multipliers that coat the floor with bacteria. As the film of mop water dries, these bacteria are ready for scuffing into the air. Ultimately, air-borne bacteria impinge on the refrigeration coils or are trapped in the humidifying water in the air conditioning machine, where they grow and multiply. Investigation of a fog room conditioner, for example, revealed its insides to be coated with a layer of material resembling Camembert cheese. This turned out to be a culture of *Proteus* and *Staphylococcus*. Whenever this machine was idle this layer dried and flaked. These flakes became air-borne when the machine was put back into commission. Air-conditioning machines must be suspect as multipliers from which bacteria are unpredictably fed into the ventilating air. Such machines should be cleansed periodically with detergent germicides to eliminate this feedback.

Control of environmental sepsis depends upon breaking up the five components of the ventilation vortex. A hygienic environment is basic to safe care of patients.

TABLE I  
*Range of Bacterial Density in Samples from 24 Boston Hospitals*

Organisms in sample	Number of samples		
	Coliforms	Staphylococci	Total count
0	83	31	5
1-50	17	51	34
51-100	1	3	11
101-150	0	1	8
151-200	1	2	6
201-250	0	2	3
251-300	0	3	3
Too numerous to count	4	9	33
Discarded because of technical difficulty	1	5	5
Total (excluding those discarded)	106	102	103
Per cent of total positive	21.7	69.6	95.1
95% confidence interval	14.2-30.3	60.0-78.3	

It can be attained by systematic cleaning by trained teams. Cleaning time must be scheduled and respected by doctors and nurses alike. Cleaning is an important constituent of therapy, and, just as no patient is too ill to receive a hypodermic medication, he is not too ill to have his environment made safe for his continuing occupancy. Unfortunately, noisy mechanical devices such as scrubbing machines and vacuum cleaners are essential to successful cleaning. Mops and brooms are ineffective and hazardous. The annual cost of keeping a hospital safely clean is 10 days' receipts per bed or 85 cents per square foot. This is far cheaper than the cost of caring for septic patients.

It is paradoxical that communities that provide bacteriological control of milk, water, restaurant service, and foodstuffs permit the operation of hospitals without bacteriological surveillance. The following are the minimal bacteriological criteria that should be enforced in hospital practice: floor count, operating room, 0 to 5/sq. cm.; floor count, ward 5 to 10/sq. cm.; air, operating room, 5 to 10/cu. ft.; air, ward, 10 to 20/cu. ft.; bed rail, 5 to 10/sq. cm.; soap, sterile; bedside carafe, sterile; and bedding, 2 mm./zone inhibition.

#### SUMMARY

The source of environmental sepsis is the septic patient who sheds bacteria. Isolation of this patient is imperative. Multipliers, spreaders, and reservoirs in every environment must be identified and controlled to provide hygienic air.

#### BIBLIOGRAPHY

1. WALTER, C. W. W.; RUBENSTEIN, A. D.; KUNDSIN, R. B., AND SHILKRET, M. A.: Bacteriology of the bedside carafe, *New England J. Med.* 259, Dec., 1958. In press.

# Cause, Prevention, and Treatment of Staphylococcal Infections in the Hospital

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This study of staphylococcal infections in surgical hospital wards is based on 52 strains of staphylococci, coagulase-positive, isolated from pyogenic infections and 19 strains obtained from members of the hospital personnel—surgical, medical, nursing, and housekeeping staff. Among the latter, 10 strains were coagulase negative and were discarded.

Last year, one of us<sup>1</sup> reported that there is no evidence that the *Staphylococcus aureus* isolated from nasal carriers among hospital personnel is the same lesion-producing organism that we find in surgical pyogenic infections. We have seen in the past year two sporadic outbreaks of staphylococcal infection, with a serious form of staphylococcal disease (pneumonia) in association with viral influenza. Persons harboring parasites were often seen before and during the influenza epidemic, but the disease itself was rare previously. This means that the outbreak of an infection among a group of persons (on the surgical ward) must be regarded as an indication that something has been going wrong (since the viral influenza epidemic) with the equilibrium between parasites and host that previously existed. If this is so, our aim should be to discover whether the microbe virulence or the host resistance, or both are implicated. We must also learn (1) if the lesion-producing staphylococci are necessarily more virulent and have a more enhanced pathogenicity for man than the more virulent of our actual strains; (2) if they have the ability to initiate new diseases in new hosts, and (3) if they are different from the staphylococcal strains isolated from healthy nasal carriers. We know today that when a lesion-producing *Staphylococcus* is introduced from outside (the nurse's or a new patient's respiratory or cutaneous infection) in a hospital ward, where there are gathered many susceptible hosts, the equilibrium between hospital patients and ubiquitous *Staphylococcus* becomes unbalanced and the diseases break out.

This paper presents a picture of the status of staphylococcal infections in our hospital, their actual importance, epidemiological study, and antimicrobial therapy. We have not studied the magnitude of the problem of staphylococcal infection as encountered at Pasteur Hospital but have been concerned with some problems involved in evaluating the role of nasal carriers between members of the hospital personnel and the effectiveness of antibiotic synergism in the management of severe staphylococcal infections.

Cause and prevention are two related aspects of epidemiological interest; treatment concerns antibiotic susceptibility and host resistance; and all depend on the persistence of organisms and the bacterial virulence.

## MATERIAL AND METHODS

My associates and myself have studied 71 strains of *Staph. aureus* obtained from in- and outpatients and from members of the hospital staff. The inpatients' strains come from sources such as: operative wound infections, urinary tract infections, secondarily infected burns, subdiaphragmatic abscess, and postoperative staphylococcal pneumonia.

The outpatients' strains (20) were mostly from cutaneous infections, some soft-tissue infections (gluteal abscesses), breast abscess, and 1 case of osteomyelitis. Almost all the staphylococci obtained from hospital personnel were nasal strains; only two were isolated from pyogenic lesions.

We divided the patients into those who had the infection at the time of admission to the hospital and those in whom the infection was probably acquired in the hospital.

Among the infections acquired outside of the hospital, there were only two initially serious infections: 1 case of osteomyelitis and 1 case of stab wound peritonitis. The other 18 apparently less serious infections present on admission (furuncles, felons, carbuncles) were not only important because of their prolonged morbidity but were even more serious than those of the next group because they occurred in patients with an impaired resistance to infection (diabetes, renal failure, impaired circulation).

Among the infections acquired in the hospital, there were 6 listed as serious, and the other 26 as less serious. The 6 severe infections included: 1 patient with a subdiaphragmatic abscess occurring after a clean laparotomy; 1 with staphylococcal pneumonia; 3 with severe secondary infection of operative wounds; and 1 case of extensive carbuncles and infections of soft tissue in a diabetic man. The other 26 patients, classified as less severe, included: secondary infections of primarily clean surgical wounds and of burns, gluteal abscess, and multiple furuncles.

Among the 19 strains obtained from members of the hospital personnel, 15 were from healthy nasal carriers, 2 from the nasopharynx of personnel with viral influenza infection, and two from pyogenic infections ( 1 felon and 1 hidrosadenitis). Among these 19 strains, 9 were pathogenic staphylococci, coagulase- and fibrinolysin-positive, 10 were coagulase-negative. A summary of these data is shown in table I.

Among the 61 coagulase-positive staphylococcal strains, we found two lysogenic strains (12 and 32), and we isolated two phages (we called these MM12 and MM32) that we have employed for the identification of the organisms. This is not a true "typing" because we did not use the 19 "basic" phages, but from a practical point of view, our method permits us to identify the different strains in a very high proportion of cases (56 per cent) (table II). The phages were isolated by the

TABLE I  
*Sources of Infections*

Number of strains studied	71
Infections present at time of admission (outpatients)	20
Osteomyelitis (strain 32)	1
Breast abscesses	3
Peritonitis	1
Gluteal abscesses	4
Furuncles, felons, carbuncles	9
Infected burns	2
Infections probably acquired in the hospital (inpatients)	32
Postoperative subphrenic abscess	1
Infected wound and burns	21
Staphylococcal pneumonia	1
Gluteal abscesses	2
Severe infection, operative wound	3
Multiple furuncles	1
Extensive carbuncle in diabetes (strain 12)	1
Staphylococcal respiratory infections (no evidence of pneumonia)	2
Members of the hospital personnel	19
With pyogenic infections	2
With viral influenza	2
Healthy nasal carriers	15
(10 strains coagulase negative)	

TABLE II

*Phage Identification of Staphylococci Isolated from Out- and Inpatients and Members of the Hospital Personnel*

Source	Number	Coagu- lase	Fibri- nolysin	MM32*	MM12*	32/12*	Nontyp- able	Observations
Outpatients, infections present on admission	20	1 9 3 7	1 9 3 5	1 — — —	— 9 — —	— — 3 —	— — — 7	Osteomyelitis (strain 32)
Inpatients, infections probably acquired in the hospital	32	14 2 16	14 2 14	14 — —	— 2 —	— — —	— — 16	Extensive carbuncle (strain 12)
Members of the hospital personnel (19)								
With pyogenic infection	2	2	2	2	—	—	—	
With viral influenza	2	2	2	2	—	—	—	
Healthy nasal carriers	15	2 3 —	2 3 —	— — —	2 — —	— — —	— 3 10	

\* MM32 = strain lysed by phage 32; MM12 = strain lysed by phage 12; 32/12 = intermediate forms.

Fisk<sup>3</sup> method and propagated on susceptible strain by the Blair<sup>4</sup> technique; the titration gave a "test dilution" of 1:100.

*Cause.* The 61 coagulase-positive *Staphylococcus* strains were classified into four groups, with regard to the source of the strains: (1) outpatients' strains; (2) inpatients' strains; (3) hospital personnel strains from healthy nasal carriers; and (4) hospital personnel strains from actual infections. Table II shows our findings. Employing the two phages isolated by us, we can identify 35 strains (57.3 per cent). Among 20 outpatients' strains, we found nine of phage type MM12, three intermediate strains 32/12, and only one of phage type MM32. Most of the inpatients' strains were of phage type MM32, and only two were classified as MM12. These figures show that lesion-producing staphylococci are different in the community and in the hospital. We do not speak of the prevalence in the hospital of particular strains of increased pathogenicity for man. This is another problem. When we studied the strains isolated from the members of the hospital personnel we found that (1) healthy nasal carriers had no typed strains or organisms phage typed MM12; (2) strains from pyogenic infections were of phage type MM32; and (3) strains from noses of hospital personnel with actual virus influenza were of phage type MM32.

The *Staphylococcus* causing our severe infections was a strain lysed by phage MM32 (approximately 43.7 per cent of postoperative wounds and subphrenic infections, pneumonia, and other staphylococcal respiratory infections). The staphylococci found in hospital personnel nasal carriers were lysed by phage MM12.

The fact that a healthy member of the hospital personnel is a nasal carrier of staphylococci does not mean that he may be the source of an organism that is capable of producing an infection in a surgical patient. It is apparent that healthy nasal carriers of staphylococci played a very small role in the spread of the staphylococcal infections in our surgical hospital. We found, also, that repeat nasal cultures on the positive carriers were not uniformly consistent with the original culture, and we have found significant changes when there was a viral influenza infection. When there is a pyogenic infection (mostly cutaneous) in a hospital

member, the origin of this infection may be produced through direct contact with one infected patient, or may be from outside, but it is always acquired by direct contact. We have not found significant differences between these two sources of contamination. Barber and Burston<sup>5</sup> recently re-emphasized the fact that the staphylococci prevalent in the hospital have, in terms of cross infection and of ability to produce a more serious staphylococcal disease, an increased pathogenicity for man. We think that, independently of its source, a lesion-producing *Staphylococcus* even though not necessarily more virulent than the *Staphylococcus* of the nasal carriers, undoubtedly has a capacity to initiate new disease, and in this sense is more dangerous.

There are for us two important means of contamination by staphylococcal disease: air-borne (in nasal carriers with virus influenza) and direct contact (in pyogenic infections). In both instances, as soon as the infection is noted the members of the hospital staff must be taken off duty, but this concerns control of the staphylococcal disease.

*Prevention.* This is an epidemiological problem, and we must determine in every case the origin of the organism pathogenic for patients and its actual method of spread. We must work on a bacteriological basis, and the surgeons must understand the necessity for routine cultures on all patients with infections of any type. The necessity for meticulous technique in all departments must be strongly emphasized, and the immediate results will be correction of faulty hospital techniques. Our efforts to control hospital staphylococcal infections are directed toward both direct contact and air-borne routes of transmission.

Direct contact may be from patient to patient directly or through hospital personnel. It would be impracticable for us to isolate hospital patients with staphylococcal infections. We have called the attention of the entire hospital staff to the importance of a meticulous dressing of all infected patients, and we have em-

TABLE III  
*Phage Identification of Coagulase-Positive Staphylococci Isolated from Related Sources*

Case no.	Strain no.	Patient	Source	Interval between first and subsequent cultures, days	Phage
14	14	Outpatient	Gluteal abscess	—	MM12
	14 a		Furuncle	20	MM12
	14 b		Inguinal furuncle	25	MM12
17	17	Outpatient	Gluteal abscess	—	32/12
	17 a		Hidrosadenitis	15	32/12
26	36	Inpatient	Operative wound infections	—	MM32
	36 a		Postoperative pneumonia	3	MM32
30	40	Inpatient	Operative wound, first post-operative dressing	—	MM32
	40 a		Subdiaphragmatic abscess	10	MM32
	40 b		Gluteal abscess	18	MM32
38	42	Inpatient	Operative wound, first post-operative dressing	—	MM32
	42 a		Respiratory infection*	6	MM32
43	47	Outpatient	Carbuncle	—	MM12
	47 a		Felon	22	MM12
61	65	Outpatient	Carbuncle	—	32/12
	65 a		Felon	25	32/12

\* Fever, cough, purulent sputum, *Staph. aureus* positive on culture, without evidence of pneumonia.

TABLE IV

*Résumé of the Most Serious Cases (22) of Staphylococcal Diseases Treated with Combination Oleandomycin-Tetracycline*

Diagnosis	First treatment*	No. cases	Daily dosage, mg.	Duration treatment, average, days	Results, per cent cured
Postoperative infections			750 to		
Wound infected (first dressing)	P S	10	1000	8	100
Subphrenic abscess	P S	1	1250	7	100
Staphylococcal pneumonia	P	1	1250	5	100
Respiratory infections	P	2	1000	9	100
Severe infections of wounds	O	3	1250	14	100
Bone infection					
Osteomyelitis		1	1250	12	100
Skin infections					
Extensive carbuncle in diabetic with renal failure		1	1000	8	100
Carbuncles in diabetic patients		3	750 to 1000	6	100

\* P = penicillin; S = streptomycin; O = oxtetracycline.

phasized the need for hand-washing and alcohol rinses by the hospital personnel after direct contact with these patients.

We think that ward dressing techniques used by Caswell and associates,<sup>6</sup> in which all dressing and instruments are sterilized by steam before cleansing by supply room personnel, are very good measures, and we shall use them. Every person who has a cutaneous staphylococcal infection is immediately taken off duty and is not allowed to return until all drainage has ceased.

The air-borne route is very important in the operating room, where there should be a minimum of visitors or students, who should remain silent and wear masks (changed every hour) and appropriate clothing and shoes (changed before entering). The local prophylactic installation of antibiotics in surgical wounds as a routine procedure has been suggested. We consider the use of drugs prophylactically as impracticable because of the necessity for protecting the patient against several organisms in addition to staphylococci for a long period of time.

*Treatment.* Treatment is concerned with antibiotic susceptibility of bacteria and with host resistance.

ANTIBIOTIC SUSCEPTIBILITY DETERMINATION. Test tube susceptibility tests were performed with each strain. The organisms were taken from an overnight broth culture. The tests were incubated for 24 hours at 37 C. and susceptibility determined by lack of turbidity in the tube. On testing oleandomycin, we also used the determination of the maximum number of cells inhibited "by an arbitrarily selected concentration of drug," as suggested by Hobby and Lenert,<sup>7</sup> instead of the determination of the minimal inhibitory concentration, and we also used the Lederberg<sup>8</sup> replica plating technique to determine the delayed bactericidal and the persistent bacteriostatic action of this antibiotic.<sup>9</sup> With these multiple in vitro studies we have a more accurate evaluation of the antibiotic susceptibility of organisms. And this is important because in the treatment of our patients we used a combination of antibiotics (oleandomycin plus tetracycline), and we know that the minimum inhibitory dosages have been criticized<sup>10</sup> on the basis that they presuppose a linear relation between inhibitory action of each drug and its dosage. The results obtained

TABLE V

*Antibiotic Resistance, u. or  $\mu\text{g.}/\text{ml.}$ , to 61 Strains Staph. aureus Coagulase Positive Isolated from Patient Infections or Members of the Hospital Personnel*

Antibiotic	Phage	No.	Resistance, per cent
Penicillin	MM 32	19	91
	MM 12	13	56
	32/12	3	100
	Nontypable	36	68
Streptomycin	MM 32	19	94
	MM 12	13	63
	32/12	3	100
	Nontypable	36	76
Tetracycline	MM 32	19	82
	MM 12	13	50
	32/12	3	33
	Nontypable	36	65
Erythromycin	MM 32	19	85
	MM 12	13	40
	32/12	3	33
	Nontypable	36	60
Oleandomycin	MM 32	19	5
	MM 12	13	0
	32/12	3	0
	Nontypable	36	2
Novobiocin	MM 32	19	10
	MM 12	13	0
	32/12	3	33
	Nontypable	36	8

are summarized in table IV. We found differences between the three groups of strains (MM12, MM32, and nontypable strains) with respect to antibiotic susceptibility. It was found that the phage type MM32 had a high percentage of resistance to penicillin, streptomycin, tetracycline, and erythromycin. The nontypable group approached this degree of resistance, while the organisms lysed by phage MM12 were less resistant, (table V). We have found only two strains resistant to oleandomycin ( $10\mu\text{g.}$ ) (3.27 per cent).

Host resistance is a very important problem and it must be studied extensively, but not here. Gamma globulin therapy in addition to antibiotics has been emphasized recently, and serum properdin levels undoubtedly have an important role as a factor in natural resistance to infection. Our studies on the properdin system will be published soon.<sup>11</sup>

All our patients were treated with a combination of oleandomycin and tetracycline,\* since previously published laboratory evidence and clinical experience proved that this combination possessed an enhanced antimicrobial action against *Staph. aureus*.<sup>12</sup>

We have treated 52 patients and 2 members of the hospital personnel with staphylococcal diseases, varying in age from 12 to 72 years. The oleandomycin-tetracycline combination was given in a total daily dosage of 750 to 1250 mg. orally in divided doses over a treatment period ranging from 3 to 16 days. In table IV are listed the most serious cases treated. For postoperative infections almost all the patients were treated first with penicillin alone or penicillin-streptomycin, and when the bacteriological studies confirmed the presence of *Staph.*

\* The trade name of Chas. Pfizer & Co. for a combination of oleandomycin and tetracycline is Signemycin.

*aureus*, oleandomycin-tetracycline was substituted with very good results. Three patients with severe postoperative wound infections were treated for three days with oxytetracycline. At this time we confirmed the presence of *Staph. aureus* resistant to penicillin, streptomycin, and oxtetracycline and therefore changed the treatment. Oleandomycin-tetracycline therapy produced an adequate clinical response in a few days with control of drainage. The most significant case was a subphrenic abscess occurring 10 days after a relatively clean laparotomy (gastro-duodenectomy for duodenal ulcer). The day of the operation a member of the hospital personnel who had viral influenza was in the operating room. He was later examined and confirmed as a nasal carrier of *Staph. aureus*, phage type MM32. The patient, who had been treated since onset of the febrile symptoms with penicillin-streptomycin, was operated on. Culture of pus obtained at operation showed staphylococci, MM32 positive. Too early removal of the drainage tube provoked a recurrence of febrile symptoms and a new subdiaphragmatic condition, which was treated and cured by oleandomycin-tetracycline therapy for seven days at a dosage of 1250 mg. daily.

#### CONCLUSIONS

1. A study has been made of 71 strains of staphylococci isolated from patient infections and nasal carriers of hospital personnel.
2. The staphylococci were identified with two phages isolated from lysogenic strains.
3. Healthy nasal carriers among hospital personnel played an insignificant role in infection.
4. Members of the hospital personnel with viral influenza were nasal carriers of more virulent and pathogenic strains.
5. The routes of transmission of organisms in surgical infections were mainly air-borne (in viral influenza) and by direct contact (in pyogenic infections).
6. All the patients (52 cases) with surgical infections were treated with a combination of oleandomycin-tetracycline with very good results.
7. Among these patients were several serious staphylococcal infections (operative wound infections, subphrenic abscess, respiratory staphylococcal infections, carbuncles in diabetic persons, and other serious illnesses).

#### BIBLIOGRAPHY

1. MORADOR, J. L.: Antibióticos y cirugía, Symposium on Antibiotics, Hospital Durand, Buenos Aires, December, 1957.
2. MORADOR, J. L.; SALDAÑA TATE, L., AND MORADOR, S.: Las infecciones estafilocóccicas en el Hospital Pasteur. Fago identificación de las cepas responsables. An. Ateneo de Clín. Quir. 3:130-135, 1958.
3. FISK, R. T.: Studies on staphylococci; occurrence of bacteriophage carrier among strains of *Staph. aureus*, J. Infect. Dis. 71:153-160, 1942.
4. BLAIR, J. E., AND CARR, M.: The bacteriophage typing of staphylococci, J. Infect. Dis. 93:1-13, 1953.
5. BARBER, M., AND BURSTON, J.: Antibiotic resistant staphylococcal infection: a study of antibiotic sensitivity in relation to phage typing, Lancet 2:578-583, 1955.
6. CASWELL, H. T.; SCHRECK, K. M.; BURNETT, E.; CARRINGTON, E. R.; LEARNER, N.; STEEL, H.; TYSON, R., AND WRIGHT, W. C.: Bacteriological and clinical experiences and the method of control of hospital infections due to antibiotic resistant staphylococci, Surg., Gynec. & Obst. 106:1-10, 1958.
7. HOBBY, G. L., AND LENERT, T. F.: Observations on the mode of action of oleandomycin, Antib. & Chemo. 8:219-227, 1958.
8. LEDERBERG, J., AND LEDERBERG, L. M.: Replica plating and indirect selection of bacterial mutants, J. Bact. 63:339-406, 1952.

9. MORADOR, J. L., AND SALDAÑA TATE, L.: Sobre el modo de acción de la oleandomicina y la spiramicina. Estudio in vitro, presented before Ateneo de Clínica Quirúrgica, Aug. 12, 1958. In press.
10. JAWETZ, E., AND GUNNISON, J. B.: An experimental basis of combined antibiotic action (report to the Council on Pharmacy and Chemistry of the A.M.A.), J.A.M.A. 150:693, 1952.
11. FERRARI, J. P.; CASTIGLIONI, R., AND MORADOR, J. L.: Determinación de los valores de la properdina en el suero en relación con los diferentes procesos patológicos. In preparation.
12. MORADOR, J. L., AND SALDAÑA TATE, L.: Treatment of fifty-two cases of infections caused by coagulase-positive staphylococci with a combination of oleandomycin and tetracycline. In: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, pp. 702-707.

# In Vitro Response of Clinical Isolates at Cook County Hospital to Antimicrobial Drugs: A One-Year Survey

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In vitro tests to determine microbial susceptibility to antibiotics and other drugs have become a major function of most hospital bacteriology laboratories. Many reports have appeared in the literature concerning this function, some having to do with methods used, some with in vitro-in vivo correlation, and others with resistance-sensitivity patterns of various microorganisms. This paper concerns the last of these three aspects and consists of data gathered from routine laboratory findings. The analysis of these data was undertaken in order to gain insight into the over-all in vitro response of large numbers of clinically important organisms as routinely isolated from a large municipal hospital. A total of 5232 sensitivity tests performed from June, 1957, to June, 1958, form the basis of this report.

## METHODS

Due to the large numbers of sensitivity tests routinely performed at this hospital, it is necessary to rely on the paper disc procedure. Each organism to be tested is isolated in pure culture, identified, and inoculated into a tube of brain-heart infusion broth prior to testing. The latter is incubated at 37C. until faint turbidity is seen, usually after about four hours (for sulfonamide testing, brain-heart infusion broth without para-aminobenzoic acid is used). One ml. of this young broth culture is then diluted 1:5 with sterile distilled water, and a swab, moistened in this diluted culture, is streaked evenly over the surface of an agar plate. For sulfonamide testing, Mueller-Hinton agar, with or without added blood, is used. For other drugs, Penassay agar (Difco) or sensitivity medium C agar (Case Laboratories) has been used. Appropriate discs are then placed on the surface of the inoculated plate and the plates incubated overnight at 37C. Readings are made according to the presence or absence of a zone of inhibition around a disc, not by the size of the zone.

The drugs routinely used at Cook County Hospital for in vitro testing vary with the organism being tested. For gram-positive bacteria, penicillin (1.5 and 10 units), erythromycin (10 and 50  $\mu\text{g.}$ ), tetracycline (10 and 50  $\mu\text{g.}$ ), and chloramphenicol (10 and 50  $\mu\text{g.}$ ) are used. For gram-negative bacteria, tetracycline, chloramphenicol, and streptomycin (10 and 100  $\mu\text{g.}$ ) are used. Nitrofurantoin (100  $\mu\text{g.}$ ) and sulfisoxazole (300 or 500  $\mu\text{g.}$ ) are used for all organisms isolated from urinary tract infections, whether gram-positive or gram-negative, in addition to the regular disc. Results are reported as sensitive or resistant or moderately resistant when two concentrations of a drug are used.

## RESULTS

Table I shows the type of specimen from which the organisms were isolated. It is obvious that the majority came from urines and local lesions (pus, burns). No attempt was made to correlate sensitivities for any one organism with its source of isolation.

TABLE I  
Distribution of Organisms According to Type of Specimen from Which  
They Were Isolated

Organism	Urine	Blood	Cerebro- spinal fluid	Throat or sputum	Body fluids	Miscel- laneous (pus, wounds)	Stool	Total
<i>Escherichia coli</i>	909	103	16	75	12	166	—	1281
Enteropathogenic <i>Escherichia coli</i>	—	—	—	—	—	—	190	190
<i>Aerobacter aerogenes</i>	159	8	—	16	1	23	—	207
Paracolon	87	11	2	2	—	19	—	121
<i>Klebsiella</i>	1	3	—	19	3	4	—	30
<i>Proteus mirabilis</i>	155	19	3	5	1	82	—	265
<i>Proteus morgani</i>	66	3	—	2	—	10	—	81
<i>Proteus vulgaris</i>	14	6	—	—	—	2	—	22
<i>Pseudomonas aeruginosa</i>	170	8	1	19	5	94	—	297
<i>Pseudomonas</i> species	191	17	9	25	3	70	—	315
<i>Salmonella</i>	2	4	—	—	—	—	35	41
<i>Hemophilus influenzae</i>	—	2	23	1	1	2	—	29
<i>Staphylococcus aureus</i> (coagulase-positive)	99	118	56	346	52	1256	5	1932
Enterococci	255	59	18	7	7	75	—	421
Total	2108	361	128	517	85	1803	230	5232

Table II shows the response of various individual groups of gram-negative organisms to the drugs used, and table III is a summary of these data. Tables IV and V show comparable data for gram-positive organisms.

Certain bacteria are not included in the routine testing program and others were tested in too few numbers to be of interest. Thus, certain groups of bacteria routinely isolated from clinical material are missing from these data.

It is obvious from analyzing these data that much variation in response to the drugs tested is exhibited by these clinical isolates. In many cases a prediction of a drug of choice for therapy would be very difficult without an in vitro test. In the gram-negative group neomycin is the drug of choice for enteropathogenic *Escherichia coli* and polymyxin is singularly effective against *Pseudomonas* organisms. Against the coliform group, in general, nitrofurantoin seemed to possess the greatest activity of all the drugs tested. The *Proteus* group of bacteria is the enigma both clinically and in vitro, although certain strains are sensitive to certain drugs. In the gram-positive group the enterococci were most sensitive to chloramphenicol and nitrofurantoin, with erythromycin also being quite active. The staphylococci in this hospital are largely resistant to penicillin, with erythromycin, chloramphenicol, and nitrofurantoin showing the greatest activity against the 1932 strains tested (only 104 strains were tested against nitrofurantoin). Tests against some of the newer drugs, such as novobiocin, oleandomycin, and ristocetin, were not done routinely because these antibiotics were not available on a routine basis at this hospital during the period covered by this study.

#### DISCUSSION

The value of an in vitro sensitivity test as a guide for the therapy of infections will depend in large measure on how the test is done and how it is interpreted. This is well illustrated in a recent publication by Hoffman et al.<sup>1</sup> These authors showed that various laboratories in the same area reported divergent results when testing the same organisms by their own particular paper disc method. Their statement

TABLE II  
Response of Various Gram-Negative Bacteria to Antimicrobial Drugs

	<i>Escherichia coli</i>		Pathogenic <i>Escherichia coli</i>		<i>Aerobacter aerogenes</i>		<i>Paracolon</i>		<i>Klebsiella</i>		<i>Proteus mirabilis</i>		<i>Proteus morgani</i>		<i>Proteus vulgaris</i>		Total of all <i>Proteus</i>		<i>Pseudo-monas aeruginosa</i>		<i>Pseudo-monas</i> species		<i>Salmonella</i> species		<i>Hemophilus influenzae</i>	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<b>Tetracycline</b>																										
Strains Tested	1281		190		207		121		30		265		81		22		368		297		315		41		29	
Sensitive	566	44.2	94	49.5	66	31.9	51	42.2	20	66.7	10	3.8	7	8.6	3	13.6	20	5.4	16	5.4	105	33.3	36	87.8	26	89.7
Moderately resistant	168	13.1	19	10	28	13.5	8	6.6	6	20	45	17	5	6.2	2	9.1	52	14.1	83	28	65	20.6	3	7.3	2	6.9
Resistant	547	42.7	77	40.5	113	54.6	62	51.2	4	13.3	210	79.2	69	85.2	17	77.3	296	80.5	198	66.6	145	46.1	2	4.9	1	3.4
<b>Chloramphenicol</b>																										
Strains tested	1281		190		207		121		30		265		81		22		368		297		315		41		29	
Sensitive	749	58.5	155	81.6	99	47.8	53	43.8	14	46.7	84	31.7	17	21	2	9.2	103	28	3	1.0	42	13.3	22	53.7	28	96.6
Moderately resistant	333	26	28	14.7	69	33.3	30	24.8	10	33.3	128	48.3	18	22.2	10	45.4	156	42.4	34	11.4	49	15.6	15	36.6	1	3.4
Resistant	199	15.5	7	3.7	39	18.9	38	31.4	6	20	53	20	46	56.8	10	45.4	109	29.6	260	87.6	224	71.1	4	9.7	0	
<b>Streptomycin</b>																										
Strains tested	1281		190		207		121		30		265		81		22		368		297		315		41		29	
Sensitive	486	37.9	95	50	62	30	46	38	13	43.4	108	40.8	9	11.1	5	22.7	122	33.1	32	10.8	45	14.3	16	39	26	89.7
Moderately resistant	144	11.3	27	14.2	13	6.3	10	8.3	7	23.3	27	10.2	9	11.1	0		36	9.8	67	22.6	48	15.3	14	34.1	2	6.9
Resistant	651	50.8	68	35.8	132	53.7	65	53.7	10	33.3	130	49	63	77.8	17	77.3	210	57.1	198	66.6	222	70.4	11	26.9	1	3.4
<b>Nitrofurantoin</b>																										
Strains tested	899				159		87		152		66		13				231		170		184					
Sensitive	757	84.2			128	80.5	50	57.5	70	46	19	28.8	6	46.2	95	41.1	1	0.6	43		23.4					
Resistant	142	15.8			31	19.5	37	42.5	82	54	47	71.2	7	53.8	136	58.9	169	99.4	141		76.6					
<b>Sulfisoxazole</b>																										
Strains tested	899				159		87		152		66		13				231		170		184					
Sensitive	276	30.7			25	15.7	21	24.1	55	36.2	5	7.6	2	15.4	62	26.8	20	11.8	48		26.1					
Resistant	623	69.3			134	84.3	66	75.9	97	63.8	61	92.4	11	84.6	169	73.2	150	88.2	136		73.9					
<b>Neomycin</b>																										
Strains tested			176																							
Sensitive			169	96																						
Resistant			7	4																						
<b>Polymyxin</b>																										
Strains tested																										
Sensitive																										
Resistant																										

TABLE III  
*Over-all Response of Gram-Negative Bacteria to Antimicrobial Drugs*

	Tetracycline	Chloramphenicol	Strepto- mycin	Nitrofurantoin	Sulfi- soxazole
Total number of organisms tested	2879	2879	2879	1730	1730
No. sensitive	1000	1268	943	1074	452
% sensitive	34.7	44.0	32.8	62.1	26.1
No. moderately resistant	434	725	368		
% moderately resistant	15.1	25.2	12.8		
No. resistant	1445	886	1568	656	1278
% resistant	50.2	30.8	54.4	37.9	73.9

that an educated guess is probably as good as a disc test for guiding therapy is debatable. It is true that in many cases an in vitro test is not needed to select the proper therapeutic agent, but here guesswork is not involved. With most of the organisms included in the present report an educated guess would lead, unfortunately, to many treatment failures. It is our opinion that the disc method has a definite place in in vitro programs, especially in the busy laboratory or in the under-

TABLE IV  
*Response of Gram-Positive Bacteria to Antimicrobial Drugs*

	<i>Staphylococcus aureus</i> (coagulase positive)		Enterococci	
	No.	%	No.	%
Penicillin				
Strains tested	1932		421	
Sensitive	423	21.9	92	21.9
Moderately resistant	231	12	72	17.1
Resistant	1278	66.1	257	61
Erythromycin				
Strains tested	1932		421	
Sensitive	1374	71.1	259	61.5
Moderately resistant	218	11.3	90	21.4
Resistant	340	17.6	72	17.1
Tetracycline				
Strains tested	1932		421	
Sensitive	796	41.2	191	45.4
Moderately resistant	609	31.5	64	15.2
Resistant	527	27.3	166	39.4
Chloramphenicol				
Strains tested	1582		357	
Sensitive	1307	82.6	286	80.1
Moderately resistant	179	11.3	63	17.7
Resistant	96	6.1	8	2.2
Nitrofurantoin				
Strains tested	104		216	
Sensitive	101	97.1	188	87
Resistant	3	2.9	28	13
Sulfisoxazole				
Strains tested	87		216	
Sensitive	16	18.4	9	4
Resistant	71	81.6	207	96

TABLE V  
*Over-all Response of Gram-Positive Bacteria to Antimicrobial Drugs*

	Peni- cillin	Erythro- mycin	Tetra- cycline	Chloram- phenicol	Nitro- furantoin	Sulfi- soxazole
Total number of organisms tested	2353	2353	2353	1939	320	303
No. sensitive % sensitive	515 21.9	1633 69.4	987 41.9	1593 82.2	289 90.3	25 8.3
No. moderately resistant % moderately resistant	303 12.9	308 13.1	673 28.6	242 12.5		
No. resistant % resistant	1535 65.2	412 17.5	693 29.5	104 5.3	31 9.7	278 91.7

staffed laboratory where the tube dilution technique is not mechanically feasible. We feel that the real problem concerning the disc method is a need for standardization of methods and interpretation of results. Until this is accomplished, variation among laboratories will occur. This indicates a need for more work, not an abolition of paper discs.

Results such as those presented here tend to serve two purposes. First, they give an over-all picture of bacterial sensitivity and resistance to drugs in use at any one institution. This may be of value in helping the physician to select therapy on a more rational basis before the results of sensitivity tests are available. Second, they enable workers in other institutions or other geographical areas to compare similarities or differences in bacterial responses to antimicrobial drugs. Analyses similar to the present one have been presented recently by Hasenclever,<sup>2</sup> Waisbren,<sup>3,4</sup> and Schneierson.<sup>5</sup> The geographical origins of these reports and the present one are all similar except for that by Schneierson, and it is interesting to note that he has reported a much higher percentage of penicillin-sensitive staphylococci than have the others. Also, we did not find nitrofurantoin to be nearly as effective against *Proteus* organisms as did Schneierson or Hasenclever. However, in general the bacterial response patterns in these various reports are comparable.

#### SUMMARY

A total of 5232 bacterial strains isolated from clinical material at Cook County Hospital were tested over a one year period for in vitro response to antimicrobial drugs. This testing was done as part of the routine work in the bacteriology laboratories and this report is a survey of these results.

Great variation in response to most of the drugs tested exists among the organisms studied, emphasizing the need for in vitro testing as a guide for therapy.

In order of decreasing effectiveness, the activity of the drugs against gram-negative organisms was as follows: nitrofurantoin, chloramphenicol, tetracycline, streptomycin, and sulfisoxazole. Neomycin was the drug of choice for enteropathogenic *E. coli* and polymyxin for *Pseudomonas* organisms.

For the gram-positive organisms, the order of decreasing effectiveness was: nitrofurantoin, chloramphenicol, erythromycin, tetracycline, penicillin, and sulfisoxazole, although relatively few strains were tested against the first and last drugs.

Certain well-known organisms were not included in these results because sensitivity tests are not needed for these bacteria. Also, certain newer antibiotics were

not included because they were not available on a routine basis in the hospital during the period of this study.

#### BIBLIOGRAPHY

1. HOFFMAN, R. V.; JACKSON, G. G., AND TURNER, M. P.: Reliability of antibiotic sensitivity tests as determined by a survey study, *J. Lab. & Clin. Med.* 51:873-882, 1958.
2. HASENCLEVER, H. F.: *In vitro* antibiotic sensitivity, a compilation of determinations from the SUI diagnostic bacteriology laboratory, *J. Iowa M. Soc.* 47:136, 1957.
3. WAISBREN, B. A., AND STRELITZER, C. L.: The sensitivities and cross resistances of gram-negative bacilli to antibiotics. *In: Antibiotics Annual 1956-1957*, New York, Medical Encyclopedia, Inc., 1957, pp. 648-666.
4. WAISBREN, B. A.: The sensitivity of staphylococci to antibiotics. A five-year study of sensitivity, usage, and cross resistance in a general hospital, *A.M.A. Arch. Int. Med.* 101:397-406, 1958.
5. SCHNEIERSON, S. S.: A survey of current bacterial susceptibility to antimicrobial agents. Comparisons with previous surveys, *J. Mt. Sinai Hosp.* 25:52-58, 1958.

## I. Characteristics of Strains from Hospital Personnel

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The presence of coagulase-positive staphylococci in the nose and throat of hospital personnel has been reported by a number of investigators. The relationship of these organisms to hospital-acquired staphylococcal infections, while generally accepted, is not well understood. In several instances reported in the literature, the source of a hospital epidemic, particularly in nurseries, has been traced to hospital personnel. In other instances, i.e., postoperative wound infections and pneumonia, the role of the hospital personnel is not so clear. In the studies reported here it was desired to obtain information regarding the following questions: (1) the incidence of staphylococci in the nasal flora of hospital personnel, (2) the nature of the staphylococci found in the personnel, and (3) the persistence of staphylococci in the nose of apparent carriers.

It was felt the information regarding these questions would be of value in the consideration of a program to reduce and control the occurrence of hospital-acquired infections due to staphylococci.

### PROCEDURE AND METHODS

Specimens from the nose and throat of the subjects were obtained with sterile cotton swabs. After the first group, nasal specimens only were obtained, since it was observed that the throat specimens showed a lower incidence of staphylococci than the nasal specimens. The swabs were rotated vigorously against the mucosa and were processed in the laboratory within a few hours. Blood agar plates were streaked with the swabs and then incubated at 37 C. The plates were examined after 24 and 48 hours' incubation, and the nature of the colonies recorded. Isolations were made of typical staphylococcal colonies from all plates showing this organism. The number of staphylococcal colonies was estimated and the observations graded as follows: +++, essentially pure culture of staphylococci; ++, predominantly staphylococci; +, significant number of staphylococci;  $\pm$ , occasional staphylococci; —, no staphylococci.

All cultures of staphylococci isolated were tested for coagulase activity. The coagulase-positive strains were tested for phage sensitivity and antibiotic sensitivity. The coagulase activity was determined by the slide procedure.<sup>1</sup>

The phage pattern was determined by a modification of the method of Blair and Carr,<sup>2</sup> employing 24 phages. Phage concentrations of 10 to 100 times the phage concentration giving confluent lysis were used. The antibiotic sensitivity was determined by the disc method.

### RESULTS AND DISCUSSION

The variation in the nasal flora within the groups studied was rather wide and is illustrated by the data presented in table I. These data are representative of that

TABLE I  
*Nature of Nasal Flora, Hospital B, 57 Subjects*

Grade	No. of subjects	% of subjects
+++	31	54.4
++	9	15.8
+	10	17.8
±	4	7.3
—	3	5.3
	—	
	57	

*Staph. aureus* carriers, 18 (31.6 per cent).

obtained on the four groups, all of which showed similar patterns. Fifty-four per cent of the subjects carried essentially pure cultures of staphylococci in the nose, while in an additional 16 per cent, the flora was predominantly staphylococci. Only a relatively small number were reasonably free of this organism. In table II the proportion of *Staphylococcus aureus* and *Staphylococcus albus* strains within those carrying staphylococci is shown. The data presented here are from one of the four groups but again are representative of the general pattern. Of the 99 subjects in this group, 16.2 per cent carried only *Staph. aureus* strains, while an additional 15.1 per cent carried *Staph. aureus* and *Staph. albus* strains. These results emphasize the desirability of distinguishing carefully between the two species of staphylococci in carrier studies, since unless this is done, the data have little significance in relation to infections. Of the 31 persons carrying *Staph. aureus* strains, 21 showed more than 100 colonies of staphylococci per plate. This suggests that these persons were carrying large numbers of the bacteria in their nares.

In table III the incidence of *Staph. aureus* in the nasal flora of the four groups

TABLE II  
*Variation in Staphylococcal Flora, Hospital C, 99 Subjects*

Organism	Colonies/plate		Total	%
	<100	>100		
<i>Staph. aureus</i> only	2	14	16	16.2
<i>Staph. aureus</i> and <i>Staph. albus</i>	8	7	15	15.1
<i>Staph. albus</i> only	46	20	66	66.6

TABLE III  
*Staph. aureus Carriers*

Hospital	No. tested	No. positive	% positive
A. 127 beds	58	16	27.6
B. 118 beds	57	18	31.6
C. 288 beds	99	31	31.3
D. 66 beds	30	6	20.0
	—	—	
	244	71	

is shown. The percentage of *Staph. aureus* carriers found is somewhat lower than has been reported by some investigators but is within the range generally found in groups of this type.

The antibiotic sensitivities of 66 of the 71 strains of *Staph. aureus* isolated in this study are summarized in table IV. Fifty-three strains were resistant to penicillin and 12 were resistant to penicillin, tetracycline, and streptomycin. Eight strains were sensitive to all the antibiotics tested. These results emphasize the high percentage of antibiotic-resistant strains in carrier strains, as is the case with those strains isolated from hospital-acquired infections.

In table V are summarized the phage sensitivity data. Of the 56 strains studied, 41 were lysed by at least one of the 24 phages used; 15 strains did not lyse with any of the test phages. Twenty strains were lysed by either phage 80 or 81, while eight strains were lysed by both phages. The most striking aspect of the phage

TABLE IV  
*Antibiotic Resistance of Staph. aureus Strains*

No. strains	Resistant to						
	P	S	T	E	C	None	PTS
A. 16 strains	11	3	3	0	0	2	3
B. 13 strains	11	2	0	0	0	2	0
C. 31 strains	25	12	11	0	0	4	9
D. 6 strains	6	0	2	0	0	0	0
66	53	17	16	0	0	8	12

P = penicillin; S = streptomycin; T = tetracycline; E = erythromycin; C = chloramphenicol.

TABLE V  
*Phage Sensitivity of Staph. aureus Strains*

No. of strains tested	56
No. of strains typable	41
No. of strains nontypable	15
No. lysing with phages 80 or 81	20
No. lysing with phages 80 and 81	8

TABLE VI  
*Variation in Phage Patterns, Hospital B, 13 Subjects*

Subject	Phage pattern
No	29, (52) 80/42B
Jo	(29)/6,7,42E,47,54,70,73,75 (77) 42B/42D
Mc	(29)/6,42E,42B
Ca	(29)/52, (52A) 80/42B/81
Po	6,47,53,54,77,VA4
Ja	29
Be	52 (52A) 80/42B/81
En	Nontypable
Le	Nontypable
Ol	Nontypable
Ka	(77)
La	Nontypable
Pe	Nontypable

TABLE VII

*Variation in Phage Pattern, Hospital A, 12 Subjects*

Subject	Phage pattern
Bl	29/6,42E,47,54,73,75,42B/81
Cu	29 (52) (79)/42B,47C/81
Wi	52A,79,80/42D
Co	29/6,7,42E,47,54,70,73,75,77,42B/42D/81
Ei	7/42E, (53) 77,42B,VA4/42D/(81)
Ki	47,53,54,73,75,77 (42B) VA4
Ch	7,42E (53) 77, VA4
No	42B (47C)/81
Th	3A,3B
Ko	Nontypable
Wo	6,42E,54 (77) 42B/42D/81
Ba	52,80/42B/81

patterns of the various strains was the extreme variation shown. Of all the strains studied, only 4 showed similar enough patterns to be considered possibly identical strains. This was somewhat unexpected, since it was anticipated that within groups of this type there would be a significant duplication of strains among the subjects. The variation in phage types is illustrated in tables VI and VII, which summarize the data on two of the three groups studied.

Since all these strains must be considered pathogenic, it follows that the acquired infections in a hospital at any one time could be due to a number of different strains from many different sources.

In table VIII the results of studies on a group of 10 persons in the same hospital over a period of approximately 5 months are summarized. Seven of the group showed a remarkable consistency in staphylococcal nasal flora over this period. These results suggest that a large percentage of persons harboring staphylococci in the nose maintain the state with little variation over long periods of time. These persons represent a continuing reservoir of pathogenic staphylococci in the hospital environment. It would appear that an important consideration in any program of control of hospital-acquired staphylococcal infections is the problem of this type of carrier.

TABLE VIII

*Persistence of Strains in Staphylococcal Carriers, Colonies of Staph. aureus per Plate*

Subject	Date examined				
	Nov. 15	Dec. 2	Jan. 10	Feb. 24	April 22
No	28	12	3	>100	>100*
Jo	1	3	>100	0	>100*
Mc	>100	0	5	0	0
Le	62	0	0	0	0
Ca	0	100	0	0	0
Ol	>100	80	>100	>100	>100*
Pe	(>100)†	(>100)†	(2)†	(>100)†	(>100)*†
La	>100	>100	>100	—	—
Ka	10	0‡	0	>100	60*
En	48	>100	0	100	100*

\* Phage and antibiotic patterns unchanged.

† Coagulase negative, highly pigmented strain.

‡ On antibiotic spray.

## SUMMARY

1. The incidence of *Staph. aureus* carriers among professional personnel of four hospitals ranged from 20 to 31.6 per cent.
2. The strains of *Staph. aureus* found in the test groups showed great variation, with only a few similar strains, based on phage type, found.
3. In the one group studied during a five-month period, the *Staph. aureus* strains present in the carriers remained remarkably constant.

## ACKNOWLEDGMENT

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## BIBLIOGRAPHY

1. CADNESS-GRAVES, B.; WILLIAM, R.; HARPER, G. J., AND MILES, A. A.: Slide test for coagulase-positive staphylococci, *Lancet* *1*:736-738, 1943.
2. BLAIR, E. J., AND CARR, M.: The bacteriophage typing of staphylococci, *J. Infect. Dis.* *93*: 1-13, 1953.

# A Study of Possible in Vivo Oleandomycin Resistance Increase in *Staphylococcus aureus*

## A Preliminary Report

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This investigation was made in conjunction with a clinical trial of triacetyloleandomycin† in the treatment of staphylococcal infection. This report is primarily concerned with the antibiotic sensitivities of the staphylococci isolated from these clinical trial patients. Specific attention is paid to the disc plate and tube dilution sensitivity results and the possibility of in vivo appearance of oleandomycin resistance during the course of therapy. An in vivo increase in staphylococcal drug resistance is known to occur during therapy with erythromycin<sup>1,2</sup> and with novobiocin.<sup>3,4</sup> Even though the percentage of strains showing resistance after therapy may be relatively low, the importance of such a phenomenon is obvious.

### MATERIALS AND METHODS

Cultures were obtained from the lesions of 100 patients chosen from the surgery clinics of the City of Memphis Hospitals. The character of these lesions ranged from badly infected insect bites and stitch abscesses to deep septic lacerations and large carbuncles. For more detailed information on types of lesions, triacetyloleandomycin dosages, and duration of therapy, the reader is referred to another article in this volume.<sup>5</sup>

The staphylococci isolated from these sites were subjected to a disc-plate sensitivity test employing Trypticase soy agar (BBL) with 5 per cent citrated human blood and the following concentrations of antibiotic discs: tetracycline, 30  $\mu\text{g.}$ ; chloramphenicol, 30  $\mu\text{g.}$ ; penicillin, 10  $\mu\text{g.}$ ; streptomycin, 100  $\mu\text{g.}$ ; erythromycin, 15  $\mu\text{g.}$ ; oleandomycin, 15  $\mu\text{g.}$

These same staphylococcal isolates were subjected to twofold serial tube dilution sensitivity tests using a working solution of 200  $\mu\text{g./ml.}$  of oleandomycin base. This test was performed in the standard manner using 0.5 ml. of broth diluent per tube and 0.5 ml. of a 1:10,000 dilution of an 18 hour broth culture as the inoculum. Tests were read after 24 hours' incubation, accepting the lowest dilution showing no growth as the end point or minimum inhibitory concentration (MIC). All tests were performed in duplicate.

As the therapy of these patients progressed, repeat cultures were obtained on each return to the clinic. If staphylococci were present, the series of tests was repeated on these isolates.

### RESULTS

Data assembled in table I present the disc plate sensitivity pattern for six anti-

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This study supported in part by a grant from J. B. Roerig & Co. Division, Chas. Pfizer & Co., Inc.

\* Sophomore Medical Student, University of Tennessee.

† This antibiotic was furnished by J. B. Roerig & Co.

TABLE I  
*Disc-Plate Sensitivity Patterns of 100 Staphylococcal Strains  
Originally Isolated from Human Lesions*

	Coagulase positive (74 strains)		Coagulase negative (26 strains)		Total	
	% sensitive	% resistant	% sensitive	% resistant	% sensitive	% resistant
Tetracycline	42	58	85	15	54	46
Chloramphenicol	73	27	92	8	78	22
Erythromycin	55	45	81	19	62	38
Streptomycin	58	42	85	15	66	34
Oleandomycin	99	1	96	4	98	2
Penicillin	39	61	69	31	47	53

biotics of 100 staphylococcal strains after initial isolation from human lesions. All strains were hemolytic, and 74 per cent were coagulase positive. For the most part, these organisms represent a population sample exhibiting relative antibiotic sensitivity, as opposed to so-called endemic, resistant hospital strains. Coagulase-producing staphylococci generally constitute a more antibiotic-resistant group than do the coagulase-negative organisms.<sup>6</sup> This appears to correlate with the organisms of this study except in oleandomycin sensitivity, which is quite high in both coagulase-positive and -negative staphylococci. This observation is not too surprising in the light of the recent availability of this drug.

Repeat disc plate sensitivity tests have been performed on 65 strains of staphylococci reisolated from lesions after courses of therapy ranging from 4 to 21 days. No change in sensitivity pattern was noted.

The results of the oleandomycin tube dilution sensitivity tests performed on the initial isolates are assembled in table II and demonstrate a strain sensitivity distribution similar to that reported by other investigators.<sup>10,11</sup>

Tube dilution sensitivity tests were performed on the same 65 reisolated strains previously mentioned. These results, summarized in table III, present no marked variation in distribution from that shown in table II. A more complete report will be forthcoming on completion of these clinical trials. On the basis of presently available data, however, some observations can be made. A comparison of the MIC exhibited by the organisms isolated from the same lesions before and after therapy demonstrated no consistent trend of increase in oleandomycin resistance. There was variation, of course, but this was evident only at low drug concentrations and the variation stayed within the accepted test variation of  $\pm 1$  dilution.

#### DISCUSSION

If one depended on the disc plate sensitivity test to choose a potentially effective agent to combat coagulase-positive organisms or simply staphylococci in general, the order of indicated drug effectiveness for the organisms initially isolated in this study would be as follows: oleandomycin, chloramphenicol, streptomycin, erythromycin, tetracycline, penicillin.

This list and the data in table I neither demonstrate equality of resistance nor suggest a constant pattern of cross resistance between erythromycin and oleandomycin. This observation is in agreement with other reports<sup>7-9</sup> indicating a basic difference in magnitude existing between types of cross resistance found within the erythromycin series. True cross resistance within this drug series surely exists in clinical isolates, but its occurrence falls short of a 100 per cent correlation.<sup>6</sup> High

TABLE II

*MIC Values of Oleandomycin for 100 Staphylococcal Strains Originally Isolated from Human Lesions*

	$\mu\text{g./ml.}$								
	0.39	0.78	1.56	3.12	6.25	12.5	25.0	50.0	100.0
Number of strains	28	37	20	7	4	3	0	0	1
% of total inhibited	28	65	85	92	96	99	99	99	100

level resistance appearing after in vitro manipulation, on the other hand, is almost uniformly associated with cross resistance to other members of the erythromycin series.<sup>8,9</sup> Examples of cross resistance occurring after in vitro treatment cannot, therefore, indicate a comparable level of this phenomenon in clinical isolates.

When the tube test data in table II are analyzed using an orally obtainable oleandomycin blood level of about 2  $\mu\text{g./ml.}$  as a point of division, one finds that all strains with an MIC of 1.56  $\mu\text{g./ml.}$  and less, or 85 per cent of the total, fall within the range of indicated oleandomycin effectiveness. In attempting to correlate these tube dilution results with those obtained in the disc test, it was found that the two strains exhibiting disc resistance were those presenting MIC at 100 and 12.5  $\mu\text{g./ml.}$ , respectively, the latter being one of the three inhibited at that concentration. Subsequent observations indicate that strains having 12.5  $\mu\text{g./ml.}$  MIC will be the only group containing a consistent mixture of disc sensitive and disc resistant strains. It may be only coincidence that this value is that concentration that most closely corresponds to the 15  $\mu\text{g.}$  disc strength.

The disc test indicating 98 per cent of the original isolates as oleandomycin sensitive appears to have the highest correlation with patient improvement since the drug was considered to be clinically effective in 99 per cent of the treated lesions studied. In the last analysis, the superior subject for the testing of antibiotic effectiveness should be an infected patient, but here too the end point may be subject to considerable variation in interpretation.

At least 65 patients yielded staphylococci on repeat culture after oleandomycin exposures ranging from 4 to 21 days. None of these reisolated strains demonstrated increased oleandomycin resistance, as judged by either the tube dilution or the disc plate sensitivity tests. The fact that none of these strains showed a significant rise in resistance was considered of interest primarily because of the character of the lesions involved. These were, for the most part, grossly infected or necrotic lesions involving a microbial population of considerable magnitude, circumstances con-

TABLE III

*MIC Values of Oleandomycin for 65 Staphylococcal Strains Isolated from Human Lesions after Treatment with Oleandomycin*

	$\mu\text{g./ml.}$								
	0.39	0.78	1.56	3.12	6.25	12.5	25.0	50.0	100.0
Number of strains	27	19	11	3	1	3	0	0	1
% of total inhibited	42	71	88	92	94	98	98	98	100

sidered to be of importance in the development of in vivo resistance.<sup>1</sup> The bacteriological effects of longer periods of oleandomycin therapy are still under study, a complete account of which will follow the completion of these additional clinical studies.

#### SUMMARY

1. Disc plate sensitivity patterns are presented on 100 strains of staphylococci isolated from untreated lesions. They demonstrate no consistent pattern of erythromycin-oleandomycin cross resistance.

2. Tube dilution minimum inhibitory concentrations for these 100 isolates indicate 12.5  $\mu\text{g.}/\text{ml.}$  to be the point of correlation with the disc test employing the 15  $\mu\text{g.}$  disc. These data also demonstrate that 85 per cent of the initial isolates exhibited minimum inhibitory concentrations less than 2  $\mu\text{g.}/\text{ml.}$ , therefore within the range of orally obtainable oleandomycin blood level.

3. Repeat disc plate and tube dilution sensitivity studies performed on staphylococcal strains reisolated from 65 treated lesions demonstrated no significant increase in in vitro oleandomycin resistance.

#### BIBLIOGRAPHY

1. THOMSON, E. F.; ROUNTREE, P. M., AND FREEMAN, B. M.: Observations on the sensitivity to erythromycin of *Staphylococcus aureus*. In: Antibiotics Annual 1955-1956, New York, Medical Encyclopedia, Inc., 1956, p. 63.
2. JOHNSON, S. A. M.; SCHUSTER, D. S., AND GRIMSTAD, F. A.: Clinical observations on the use of erythromycin in acne vulgaris and some pyogenic skin eruptions. In: Antibiotics Annual 1956-1957, New York, Medical Encyclopedia, Inc., 1957, p. 164.
3. FINLAND, M., AND NICHOLS, R. L.: Novobiocin, Practitioner 179:84, 1957.
4. COLVILLE, J. M.; GALE, H. H.; COX, F., AND QUINN, E. L.: Clinical observations on the use of novobiocin in penicillin-resistant staphylococcal septicemia. In: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 920.
5. LEMING, B. H., JR., AND FLANIGAN, C., JR.: A clinical evaluation of triacetyloleandomycin in the treatment of infections due to gram-positive cocci. In: Antibiotics Annual 1958-1959, New York, Medical Encyclopedia, Inc., 1959, p. 418.
6. LEMING, B. H., JR., AND FLANIGAN, C., JR.: Correlation of sensitivity patterns of oleandomycin and clinical effectiveness. In: Antibiotics Annual 1958-1959, New York, Medical Encyclopedia, Inc., 1959, p. 414.
7. ROSS, S.: PA 105, a new antibiotic: some clinical observations. In: Antibiotics Annual 1955-1956, New York, Medical Encyclopedia, Inc., 1956, p. 600.
8. NEEDHAM, G. M., AND GERACI, J. E.: Laboratory studies of oleandomycin (Matromycin), AM & CT 3:334, 1956.
9. ENGLISH, A. R.: Oleandomycin: cross-resistance studies with clinical isolates of *Micrococcus pyogenes* var. *aureus*. In: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 756.
10. ELLIOTT, H. J., AND HALL, W. H.: Activity of oleandomycin salts, mixtures, and combinations against staphylococci. In: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 716.
11. ROSS, S.; ZAREMBA, E. A., AND PUIG, J. R.: Some comments on the lack of synergism of tetracycline-oleandomycin mixture. In: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, p. 723.

# Intraperitoneal Kanamycin

## Comparison with Other Antibiotics Administered Intraperitoneally

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The intraperitoneal instillation of antibiotics has been increasingly employed for peritonitis and for operations on the large bowel<sup>3, 6-10</sup> even though relatively few critical experiments<sup>11-13</sup> have been conducted to evaluate this procedure. When kanamycin was found to be of value in the preparation of patients for surgery of the large bowel,<sup>1</sup> it was also investigated for intraperitoneal instillation in similar cases. The absence of side effects after the intraperitoneal instillation of kanamycin suggested a re-evaluation of other drugs used in comparable intraperitoneal dosage.

This report deals with a comparative study of the blood levels and the local and systemic effects obtained with the intraperitoneal instillation of tetracycline,\* neomycin,† and kanamycin,\* since these three agents would be most widely employed for peritonitis or peritoneal lavage after a colon anastomosis.

### METHODS

The antibiotics were injected intraperitoneally into unanesthetized dogs and rabbits and into anesthetized dogs in varying total dosages and varying concentrations. This study was restricted to the effects of a single intraperitoneal injection. A no. 14 needle was inserted through the abdominal wall and aspirated to determine whether the needle had punctured the intestine. Approximately 6 inches of polyethylene tube was inserted through this needle and the antibiotic solution injected through the plastic tube.

All antibiotics used were in the forms available for intramuscular injection. Most antibiotics were dissolved in 50 ml. of sterile saline and injected over a period of approximately one minute, after which the plastic tubing was removed.

The animals were observed for any immediate effects, such as pain, and for survival. Animals that died were autopsied. Animals that survived were subjected to a sacrifice laparotomy some weeks after the injection to determine whether the injections had produced any local effects.

Antibiotic serum levels were determined by the cylinder-plate technique at one or two hours and at 24 or 48 hours.

Because ether anesthesia has been reported to potentiate the toxic effects of neomycin,<sup>2, 4, 5, 14</sup> some studies were conducted on dogs anesthetized with ether.

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\* The trade name of Bristol Laboratories Inc. for tetracycline phosphate complex is Tetrex; for kanamycin, Kantrex.

† The trade name of The Upjohn Co. for neomycin is Mycifradin.

TABLE I  
*Mortality in Experimental Intraperitoneal Antibiotic Administration*

Antibiotic	Rabbits*	Dogs	Dogs with anesthesia	Rabbits	Dogs	Dogs with anesthesia
		250 mg.			500 mg.	
Kanamycin	—	—	—	—	—	—
Neomycin	0/4	—	0/5	0/5	0/4	—
Tetracycline	0/5	0/5	0/5	3/5	—	—
		1 Gm.			2 Gm. or more	
Kanamycin	—	0/7	0/5	0/10	0/4	—
Neomycin	2/5	0/5	0/5	2/2	0/5	—
Tetracycline	4/4	1/3	—	—	—	—

— = subjects not used at this dosage.

\* Number of deaths/number of animals used.

#### TETRACYCLINE

Tetracycline\* was administered in a 250 mg. dosage to 5 rabbits, only 1 of which showed toxicity in the form of a bloody peritoneal effusion one month after injection. The same dosage caused intense pain in 3 of 5 unanesthetized dogs, but no immediate effects in anesthetized dogs. At sacrifice no evidence of local toxicity was detectable in either group of dogs.

Three of 5 rabbits injected with 500 mg. died within 24 hours (table I). All showed congestion and granular deposits of antibiotic on the peritoneal surfaces. One animal that survived exhibited pain on injection, but no abnormalities were found at sacrifice in this or the remaining animal.

When the dosage was increased to 1 Gm. of tetracycline, all rabbits showed intense pain on injection, all were in clinical shock within one hour, and all died within 20 hours (table I). Autopsy showed yellowish discoloration of all abdominal viscera and pleural effusion in all animals. This dosage was fatal to only 1 of 3 dogs (table I), but all exhibited severe pain on injection. The dog that died had intense yellow discoloration of the liver. The 2 survivors were sacrificed at one month and showed numerous intraperitoneal adhesions.

Blood levels between 16 and 68  $\mu\text{g./ml.}$  of serum were found in the rabbits at one to two hours after injection (figs. 1, 2, and 3). Except for a slightly increased level in the animals receiving 1 Gm., there were no major differences between the levels observed in the other animals. For those rabbits that survived 24 hours, serum levels ranged between approximately 1 and 2  $\mu\text{g./ml.}$  of serum.

The dogs that received 250 mg. of tetracycline without anesthesia had one-hour levels averaging approximately six times as high as animals that did not receive anesthesia. The dogs that received 1 Gm. of tetracycline had levels two to four times as high as the ones that received only 250 mg. At the end of 48 hours, levels less

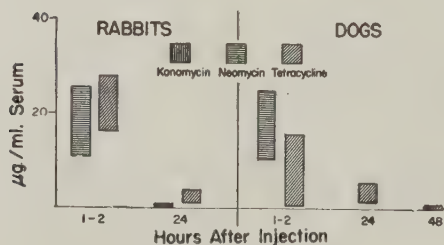
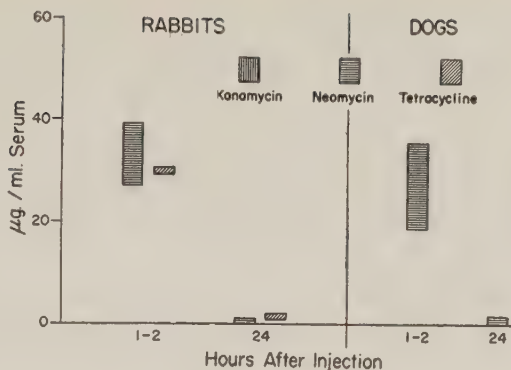


FIG. 1. Antibiotic serum levels following the intraperitoneal administration of 250 mg. of neomycin and tetracycline in rabbits and dogs.

\* All tetracycline used was tetracycline phosphate complex.

FIG. 2. Antibiotic levels in the serum following the intraperitoneal administration of 500 mg. of neomycin and tetracycline.



than 0.5  $\mu\text{g.}/\text{ml.}$  of serum were still detectable in the animals that received 250 mg. of tetracycline with anesthesia. The animals that received 1 Gm. had levels around 20  $\mu\text{g.}/\text{ml.}$  of serum, which is 100 times the level of the animals that received only a quarter of the dosage. The levels observed in the animals that received 250 mg. without anesthesia were in the neighborhood of 1 to 5  $\mu\text{g.}/\text{ml.}$  of serum at 24 hours.

These observations show that a high serum level of tetracycline rapidly follows its intraperitoneal administration, and that serum levels remain elevated for 24 to 48 hours.

#### NEOMYCIN

Neomycin was administered without any immediate or late effects as follows (table I): 250 or 500 mg. to 9 rabbits; 250 mg. to 5 anesthetized dogs; 500 mg. to 4 unanesthetized dogs; and 1.0 Gm. to 5 anesthetized and 5 unanesthetized dogs. One Gm. given to rabbits without anesthesia was lethal for 2 of 5 rabbits. Two Gm. of neomycin was lethal within one hour to both rabbits to which it was given, and produced vomiting in 4 of 5 dogs.

Serum levels in the rabbit were lower with the 250 mg. dosage than with the other dosages, but no significant differences were noted between the 1 Gm. and the 500 mg. dosage at one hour (figs. 1, 2, and 3). All but 1 of the rabbits that survived for 24 hours had less than 1  $\mu\text{g.}$  of neomycin in the serum at 24 hours. Serum levels in the dogs seemed to increase as the dosage of neomycin increased, as shown by the levels between 11 and 25  $\mu\text{g.}$  in the animals receiving 250 mg. in comparison with levels between 79 and 95 in the animals receiving 2 Gm. There was no differ-

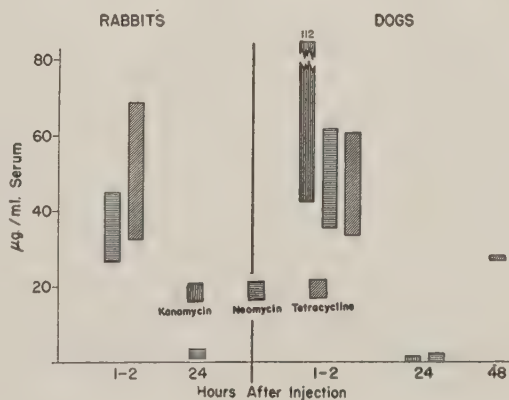


FIG. 3. Antibiotic serum levels following the intraperitoneal administration of 1.0 Gm. of kanamycin, neomycin, or tetracycline in rabbits and dogs.

ential between anesthetized and nonanesthetized dogs receiving neomycin. Blood levels at 24 hours were generally less than 1  $\mu\text{g.}/\text{ml.}$  of serum.

#### KANAMYCIN

Two Gm. of kanamycin was injected intraperitoneally to each of 10 rabbits (table I). Neither immediate nor delayed toxicity was observed in any of these animals, and the absence of toxicity with this very large dosage made it unnecessary to investigate the reaction to a lower dosage in rabbits.

One Gm. of kanamycin was tolerated without effect by 7 dogs without anesthesia and by 5 dogs with anesthesia (table I). Two Gm. was administered intraperitoneally to 2 dogs without any detectable effect. Five Gm., given intraperitoneally, caused pain in a 20 per cent solution in 1 dog but did not cause any reaction in a 10 per cent solution in another dog.

The median serum level at one hour after the injection of 2 Gm. of kanamycin in each of 10 rabbits was 54.5  $\mu\text{g.}/\text{ml.}$  of serum. The highest value was 70 and the two lowest values were 3 and 25. No 24 hour specimens were obtained in this series.

In the dogs, serum levels at one hour ranged between 42 and 112  $\mu\text{g.}/\text{ml.}$  of serum (fig. 3) with no significant difference noted between the anesthetized and nonanesthetized animals. At the end of 24 hours, most of the dogs had less than 1  $\mu\text{g.}/\text{ml.}$  of serum.

#### DISCUSSION

To be useful for routine intraperitoneal instillation, an antibiotic should provide adequate control of the bacterial flora that may be expected in the peritoneal cavity in peritonitis or after operation on the gastrointestinal tract; should produce a minimum of pain on injection; should produce a minimum of local or systemic reactions; and should have a wide margin of safety between the effective and the toxic dosages. Antibiotics instilled in anesthetized patients would not produce pain, but it seems reasonable to assume that pain is a result of serosal irritation, which subsequently may produce adhesions. Since intraperitoneal antibiotic instillation will most frequently be utilized in anesthetized patients, the possible synergistic activity of the antibiotic and the anesthetic agent should be considered.

If the antibiotic can be readily absorbed from the peritoneal cavity and produce a satisfactory blood level, this is an additional advantage to this method of drug therapy although it should not be the prime purpose for drug administration. The possible value of a prolonged elevation of serum antibiotic level from a single intraperitoneal injection remains to be evaluated.

Since the intraperitoneal administration of 500 mg. or more of tetracycline was lethal to the rabbit, this quantity should be used in patients only with caution. While the same dosage was not lethal for the dog, it did produce severe pain in both dogs and rabbits. The undesirable local reactions and the lethal effect of larger dosages combine to make tetracycline an unsatisfactory drug for intraperitoneal administration.

Neomycin produced neither local nor systemic reactions when 500 mg. or less was injected in the rabbit, but 1 Gm. was lethal to 2 of 5 rabbits, and 2 Gm. was lethal to both of the rabbits injected. When neomycin was given to dogs, no reactions were noted in dosages up to and including 1 Gm., but 2 Gm. produced vomiting within one half hour of injection.

TABLE II

*Maximum Tolerated Dosage, mg./Kg. Body Weight*

Drug	Rabbits	Dogs
Kanamycin	745	208
Neomycin	186	104
Tetracycline	93	26

Kanamycin produced neither local nor systemic toxic effect in the nonanesthetized rabbit or dog or in the anesthetized dog in a dosage up to and including 2 Gm. A 5 Gm. intraperitoneal injection was associated with pain only when given in a very concentrated solution, but produced no effects when the same quantity was given in a more dilute solution.

It cannot be said that the lower toxicity of kanamycin was related to a lower absorption rate since the serum levels of the rabbits that received kanamycin and survived were considerably higher than the serum levels observed in those animals that succumbed to intraperitoneal neomycin. Similarly, in the dog, higher serum levels were obtained with intraperitoneal kanamycin than with neomycin regardless of whether this was with or without anesthesia.

Most of the reported complications with intraperitoneal neomycin have occurred in infants or in adults where the wrong concentration of solution was inadvertently used.<sup>2, 4, 5, 9, 14</sup> In the experiments reported here, the maximum dosage tolerated without reaction has been tabulated (table II). These data indicate that both species tolerated a higher dosage level of kanamycin. On this basis, the safe dosage of either kanamycin or neomycin for patients should be well above those that are generally administered. However, the maximum apparent safe dosage is higher than that which has been reported to be lethal in patients. While it is obvious that there are species differences between the rabbit, the dog, and the human being, our own observations of intraperitoneal tetracycline and the reported difficulties with neomycin have tended to substantiate these findings.

On the basis of these observations, we believe that the intraperitoneal instillation of an effective dose of tetracycline is ill advised, and that intraperitoneal tetracycline therapy should not be used. Neomycin would appear to be safe provided the dosage is very carefully controlled. Reports in the literature indicate that mistakes will be made from time to time and that some anesthetized persons will apparently have a heightened susceptibility to the respiratory depression of neomycin. Therefore, intraperitoneal neomycin should be used only with the utmost caution. Kanamycin has thus far been administered intraperitoneally to rabbits and dogs in very large dosages without either local or systemic toxicity, without any evidence of peritoneal irritation, with satisfactory serum levels within one hour after the intraperitoneal instillation, and with minimal serum levels 24 hours later.

#### CONCLUSIONS

Tetracycline should not be instilled into the peritoneal cavity because it produces pain and adhesions and because in a large dosage it is lethal.

Neomycin should be instilled in the peritoneal cavity only with caution.

Experimental studies of the intraperitoneal injection of up to 5 Gm. of kanamycin into dogs and rabbits have indicated that this drug is safe and can be recommended for this purpose.

We would like to express our appreciation to Bristol Laboratories Inc. for kanamycin supplied in the intramuscular form and for tetracycline phosphate complex supplied in the intramuscular form, and to The Upjohn Co. for neomycin supplied in the intramuscular form.

## BIBLIOGRAPHY

1. COHN, I., JR.: Kanamycin for bowel sterilization, *Ann. New York Acad. Sc.* 76:212, 1958.
2. ENGEL, H. L., AND DENSON, J. S.: Respiratory depression due to neomycin, *Surgery* 42:862, 1957.
3. GREEN, H. G.: Intraperitoneal neomycin in the treatment of acute bacterial peritonitis, *Surg., Gynec. & Obst.* 107:169, 1958.
4. PITTINGER, C. B., AND LONG, J. P.: Danger of intraperitoneal neomycin during ether anesthesia, *Surgery* 43:445, 1958.
5. PRIDGEN, J. E.: Respiratory arrest thought to be due to intraperitoneal neomycin, *Surgery* 40:571, 1956.
6. PRIGOT, A.; MAYNARD, A. DE L., AND AVECILLA, M.: Intraperitoneal neomycin as an adjunct in the surgical management of generalized peritonitis. *In: Antibiotics Annual 1955-1956*, New York, Medical Encyclopedia, Inc., 1956, pp. 122-126.
7. POTH, E. J.: Intestinal antiseptics. Present status in surgical conditions, *West. J. Surg.* 60:205, 1952.
8. POTH, E. J.: Intestinal antiseptics, *Am. J. Surg.* 88:803, 1954.
9. POTH, E. J.: Critical analysis of intestinal antiseptics, *J.A.M.A.* 163:1317, 1957.
10. SCHATTEN, W. E.: Intraperitoneal antibiotic administration in the treatment of acute bacterial peritonitis, *Surg., Gynec. & Obst.* 102:339, 1956.
11. SCHATTEN, W. E., AND ABBOTT, W. E.: Intraperitoneal administration of terramycin in the treatment of experimental peritonitis, *Surg., Gynec. & Obst.* 97:445, 1953.
12. SCHATTEN, W. E.; DESANCTIS, A. L., AND ABBOTT, W. E.: Intraperitoneal administration of neomycin in the treatment of peritonitis: experimental and clinical observations. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc., 1955, pp. 691-698.
13. SCHATTEN, W. E.; DESPREZ, J. D.; CLINE, T. N., AND ABBOTT, W. E.: Intraperitoneal administration of terramycin in the treatment of acute bacterial peritonitis. *In: Surgical Forum*, 1953, Philadelphia, Am. Coll. Surgeons, 1954, pp. 630-637.
14. WEBBER, B. M.: Respiratory arrest following intraperitoneal administration of neomycin, *A.M.A. Arch. Surg.* 75:174, 1957.

# Antibiotic Therapy of Clostridial Myonecrosis in Irradiated Mice

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In the event of an atomic disaster in a densely populated area, a vast majority of the population will be exposed to varying degrees of irradiation. Among those receiving sublethal amounts of irradiation, some will receive concurrent wounds. Others may be wounded in subsequent military operations. Most, if not all, traumatic wounds are contaminated with various bacteria and a significant number of these contaminated wounds will become infected with members of genus *Clostridium*. Because the number of medical personnel will be limited and the casualty load large, it is important to determine the influence of sublethal irradiation on the course of clostridial myonecrosis, and the influence of self-administered antibiotics on such wounds. This study was initiated to test the practicality of using oral antibiotics for the treatment of clostridial infections in individuals who had previously received a sublethal dose of roentgen-ray irradiation.

## MATERIALS AND METHODS

*Irradiation of Animals.* Albino male mice, Bagg strain, obtained from the WRAMC colony, initially weighing from 19 to 22 Gm. were used throughout this study. Approximately 50 mice were placed in a wooden cylindrical container 14 inches in diameter and 1.5 inches deep. The mice were free to wander within the container during the irradiation process but the depth of the container prevented the animals from climbing over each other. The lid for the container was made of Lucite. There were an adequate number of perforations to supply adequate ventilation to the animals during the irradiation process. Mice were exposed to 350 r measured in air at a rate of from 31.08 to 32.43 r/minute. The radiation was supplied by means of a GE Maxitron 250 unit. The radiation factors were: 250 Kv., 30 ma, 1.0 mm. Cu, and 1.0 mm. Al with HVL of 2.14 mm. Cu, TSD 70 cm. Sham irradiated mice were kept in the same cylindrical container for the same length of time as the irradiated animals. After irradiation, the mice were placed in glass jars, 10 mice per jar. Water and food, free of antibiotics, were available ad libitum.

*Inoculum Preparation.* Five blood azide egg yolk agar plates, heavily seeded with a strain of *Clostridium perfringens*, isolated from a traumatic wound, were incubated anaerobically in a Brewer jar at 37 C. for 18 to 24 hours. The microorganisms were harvested from the agar with sterile cotton swabs and suspended in 5 ml. of physiological saline. Serial dilutions of the microorganisms were carried out in *Cl. perfringens* toxic filtrate diluted one to 10 with physiological saline until the desired turbidity was obtained. The number of viable microorganisms was estimated in each experiment by making blood agar pour plates of appropriate dilutions.

The antibiotics used were oral preparations obtained on the open market. They were oxytetracycline, penicillin V (pediatric), and chloramphenicol palmitate. The strain of *Cl. perfringens* used is sensitive in vitro to 0.63 units of penicillin, 1.25  $\mu$ g. of oxytetracycline, and 2.5  $\mu$ g. of chloramphenicol. Dilutions of the antibiotics were made in water so that the desired dosage was in 0.2 ml. The antibiotics were

TABLE I

*Effect of Total Body Roentgen-Ray Irradiation on Weight and Leukocyte Counts in Mice*

Time post irradiation, days	TBX dosage, r	White blood cell count at time of injection, number/cu. mm.	Weight at time of injection, Gm.
1	350	2650	23.7
	0	9050	23.9
4	350	1050	23.8
	0	10950	25.8
7	350	1975	24.9
	0	8825	27.5
11	350	3350	28.3
	0	8800	28.5
14	350	4200	29.9
	0	8700	30.8

administered orally by syringe and a needle covered with polyethylene (size PE90) tubing. The tubing extended one-half inch beyond the point of the needle. Antibiotic therapy was initiated four hours after the injection of bacteria and was repeated at 12 hour intervals, the last antibiotic being given 64 hours after injection of the organisms.

The tube dilution method was used to determine antibiotic levels of plasma, which was obtained by cardiac puncture. Routine procedures were used for leukocyte determinations and bacteriological studies.

## RESULTS

Initially studies were carried out to determine the effect of whole body roentgen-ray irradiation on the susceptibility of mice to this infection. The mice were irradiated as described earlier and at varying intervals injected with the organism suspended in a sublethal amount of toxic filtrate. Three roentgen-ray irradiated and 3 sham irradiated mice, chosen at random from groups to be injected were sacrificed for white cell determinations on each injection day. The leukopenia resulting from

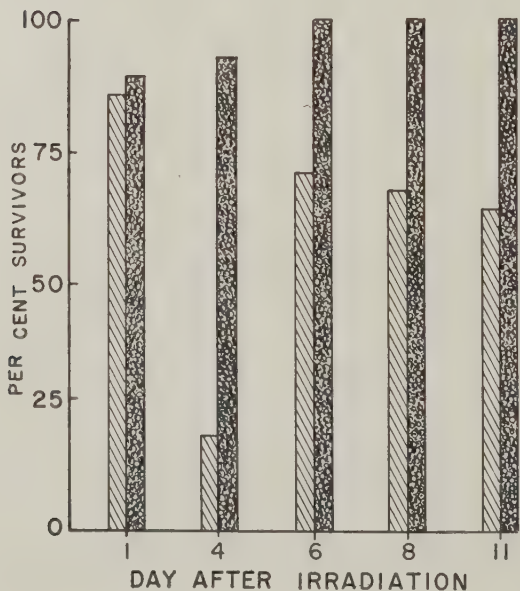
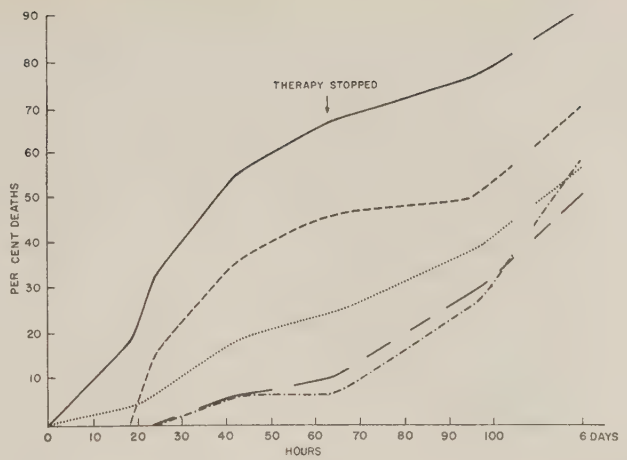


FIG. 1. Effect of 350 r of total body roentgen-ray irradiation on susceptibility of mice to *Clostridium perfringens*. Inoculum,  $10^5$  *Clostridium perfringens* suspended in a sublethal culture filtrate. ▨ roentgen-ray irradiated; ■ sham irradiated.

FIG. 2. The effect of oral penicillin V on roentgen-ray irradiated (350 r of total body) mice injected with *Clostridium perfringens*, inoculum containing  $3.0 \times 10^4$  organisms. — No therapy; - - - 600 units twice daily; ..... 1000 units twice daily; - · - · - 1400 units twice daily; — — — 1800 units twice daily.



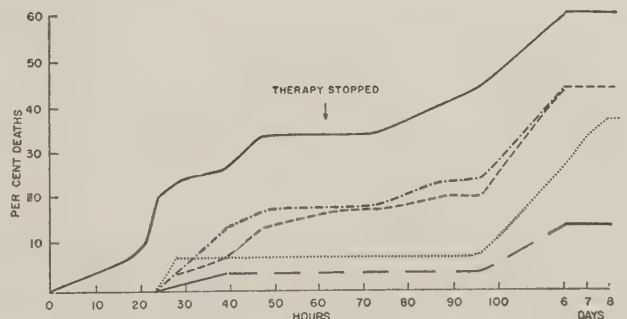
the irradiation can be seen in table I. It is apparent that there was a profound leukopenia by the fourth post-irradiation day and that the roentgen-ray irradiated mice gained weight more slowly than those that were sham irradiated. The responses of these mice to the injection of the bacteria are in figure 1. Each bar represents 27 mice. It can be seen that the greatest increase in susceptibility occurred on the fourth post-irradiation day, the time of the greatest leukopenia. This experiment was repeated with the modification of staggering the days of irradiation and challenging all the animals on the same day. The results were essentially the same. Thus, all of the animals were challenged with bacteria on the fourth post-irradiation day in the following experiments.

In figure 2 it can be seen that the higher doses of penicillin V were very effective in preventing death until therapy was discontinued. In figure 3, where oxytetracycline was used, the results are not so clear-cut as with penicillin, but again it is evident that the administration of the antibiotic prolonged survival time. When chloramphenicol was used, (fig. 4) the degree of protection was much less than with the other two antibiotics.

It was possible to recover *Cl. perfringens* from the site of injection regardless of the antibiotic used. That plus the fact the mice were no longer protected when therapy was discontinued emphasizes the point that the function of these antibiotics was prophylactic and that their use in a mass casualty situation would be to buy time, i.e., to enable a delay in the necessary surgical intervention.

The effect of total body roentgen-ray irradiation on the absorption of oral antibiotics was tested by comparing plasma levels of normal mice with the levels of mice irradiated and injected with bacteria as described earlier. The results are shown

FIG. 3. Effect of oral oxytetracycline on roentgen-ray irradiated (350 r of total body) mice injected with *Clostridium perfringens*. Inoculum contained  $1.3 \times 10^4$  organisms. — No therapy; - - - 250 units twice daily; ..... 500 units twice daily; - · - · - 750 units twice daily; — — — 1000 units twice daily.



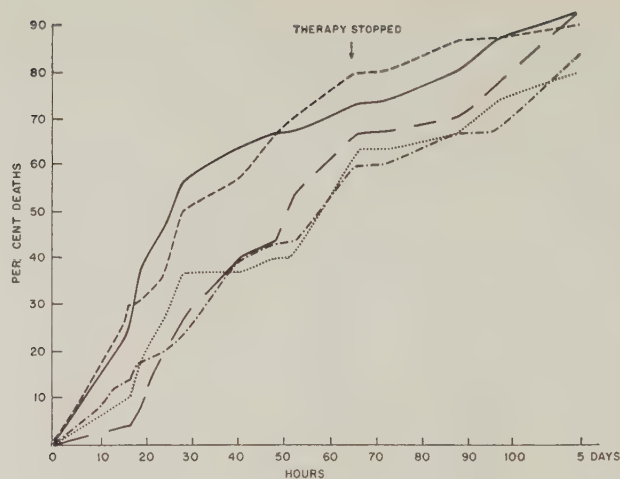


FIG. 4. The effect of oral chloramphenicol on roentgen-ray irradiated (350 r of total body) mice injected with *Clostridium perfringens*, inoculum containing  $3.0 \times 10^4$  organisms. — No therapy; ---- 250 units twice daily; ..... 500 units twice daily; -.-.- 750 units twice daily; — — 1000 units twice daily.

in table II. It is evident that 350 r had little or no effect on the plasma levels of these antibiotics.

## DISCUSSION

Most of the studies on irradiation and infection have been concerned with the invasion of the body by the endogenous organisms usually following lethal doses of irradiation. There is general agreement among investigators that the microorganisms responsible for bacteremia and infection resulting from exposure to irradiation originate from the gastrointestinal tract, because enteric organisms have been isolated from the blood and tissues of irradiated animals.<sup>1-4</sup> We were unable to culture bacteria from organs or blood of 16 irradiated mice sampled on the first, fourth and seventh post-irradiation days. However, a number of positive organ and blood cultures were found in irradiated animals following injection of *Cl. perfringens*. The organisms identified were *Cl. perfringens*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Thus the participation of endogenous organisms in the mortality of these animals cannot be discounted.

Under the conditions used, 350 r of total body roentgen-ray irradiation enhanced the susceptibility of mice to infection by *Cl. perfringens* and the time of greatest susceptibility coincided with the time of greatest leukopenia. The use of antibiotics

TABLE II  
Effect of Irradiation on Absorption of Oral Antibiotics

Antibiotic	Dose	TBX irradiation, r	Blood level, u. or $\mu\text{g.}/\text{ml.}$ ,*					
			1	2	3	4	6	12
Penicillin V	1000 u.	0	0.08	0.16	0.08	0	0	0
		350	0.16	0.16	0.08	0	0	0
	1800 u.	0	0.64	0.32	0.32	0	0	0
		350	0.32	0.32	0.32	0.16	0	0
Oxytetracycline	1000 $\mu\text{g.}$	0	0.6	1.2	0.6	0.3	0	0
		350	0.3	0.9	0.6	0	0	0
	500 $\mu\text{g.}$	0	0.3	0.6	0.45	0.3	0	0
		350	0	0.6	0.6	0	0	0

\* Mean levels based on 6 mice each. Penicillin levels in units/ml. Oxytetracycline levels in  $\mu\text{g.}/\text{ml.}$

increased the rate of survival during the period of their administration, but the antibiotics were unable to eradicate the infection. The order of the effectiveness of the three antibiotics could be correlated to the sensitivity of the organism to them. This is in keeping with the findings of Sanford et al<sup>6</sup> who found that the early administration of selected antibiotics decreased the mortality of rabbits surgically infected with various bacteria provided the wounds were primarily contaminated with bacteria that were sensitive in vitro to the specific antibiotic. The use of antibiotics in this study must be considered as providing partial prophylaxis and a subsequent delay in surgical intervention.

The finding that roentgen-ray irradiation did not interfere with the rate of absorption of antibiotics from the gastrointestinal tract of mice is in agreement with similar findings in other species. Evans,<sup>5</sup> in related studies, found that neither lethal nor sublethal irradiation interfered with absorption of penicillin V or oxytetracycline from the gastrointestinal tract of rats or dogs.

#### SUMMARY

1. A single dose of 350 r of total body roentgen-ray irradiation increased the susceptibility of mice to infection by *Cl. perfringens*. The increase in susceptibility was the greatest on the fourth post-irradiation day which was the day of greatest leukopenia.

2. Oral administration of penicillin V and oxytetracycline significantly decreased the mortality rates during the period of therapy. However, none of the antibiotics tested eradicated the infection.

3. Roentgen-ray irradiation did not interfere with the absorption of antibiotics from the gastrointestinal tract.

#### BIBLIOGRAPHY

1. BOND, V. P.: The role of infection in illness following exposure to acute total body irradiation, *Bull. New York Acad. Med.* 33:369, 1957.
2. CONARD, R. A.: Some effects of ionizing radiation on the physiology of the gastrointestinal tract: A review, *Radiation Research* 5:167, 1956.
3. HATCH, M. H.; CHASE, H. B.; FENTON, P. F.; MONTAGNA, W., AND WILSON, J. W.: Response of x-irradiated mice to intravenous inoculations of intestinal bacteria, *Proc. Soc. Exper. Biol. & Med.* 80:632, 1952.
4. MILLER, C. P.; HAMMOND, C. W., AND TOMPKINS, M.: The role of infection in radiation injury, *J. Lab. & Clin. Med.* 38:331, 1951.
5. EVANS, J. R.: Personal communication.
6. SANFORD, J. P.; EVANS, J. R.; PRENDERGAST, P. A.; BALCH, H. H., AND HUGHES, C. W.: An experimental evaluation of the usefulness of antibiotic agents in the early management of contaminated traumatic soft tissue wounds, *Surg. Gynec. & Obst.* 105:5, 1957.

The views and opinions expressed herein do not necessarily represent those of The Surgeon General, the Department of the Army, or the Department of Defense.

# Therapy of Psittacosis-Infected Parrots with Chlortetracycline

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Psittacosis-related viruses are widespread in nature.<sup>1,2</sup> While few of the psittacosis-like viruses induce clinical disease in man, a prominent zoonotic example of this group is psittacosis of avian origin. The incidence of this disease in man is related to the extent of his contact with infected birds. Such an infection in birds may induce a carrier state, with relatively poor immunity, and in which clinical relapses are related to environmental stress factors. Infected birds may shed the virus without clinical manifestations of disease. Recent surveys have shown that most of the infections in man come from contact with household birds and with commercially processed domesticated poultry, presumably through inhalation of infective aerosols.<sup>3</sup>

After the therapeutic benefit of tetracycline was demonstrated on experimentally infected animals,<sup>4</sup> its practical value was amply documented with psittacosis-related viral infections in man.<sup>5-8</sup> Multiple injections of aqueous chlortetracycline showed some therapeutic value in psittacosis-infected psittacine birds.<sup>9,10</sup> However, the labor involved and the traumatic effect of repeated injections into the same muscle were factors that limited its usefulness. A preparation of chlortetracycline in sesame oil has effectively cleared psittacosis infected parakeets of the virus.<sup>11</sup> The therapeutic dosage was determined with experimentally infected birds and subsequent therapeutic trials were run with 341 naturally infected parakeets. Two intramuscular injections two days apart, each containing 5 mg. of the drug, cleared 190 such birds of the virus; whereas 86 of 151 (56.9 per cent) of the untreated controls yielded psittacosis virus by the conventional techniques of virus isolation in mice. Recent studies show that one injection of this drug preparation (20 mg.) effectively cleared 51 parakeets of psittacosis virus, whereas virus was recovered from 25 of 43 (58 per cent) untreated controls.<sup>12</sup>

This therapeutic procedure has now been applied to a larger psittacine species of South America (*Chrysotis amizonicus*). Over a period of one and a half years, three flocks of parrots, presumed to be infected with psittacosis virus, were randomly divided and each bird from the treated group received 166 mg. of chlortetracycline hydrochloride in sesame oil per Kg. body weight, two injections into alternate pectoral muscles two days apart. Each untreated control bird was autopsied after natural death or following exsanguination within the month. The treated birds were exsanguinated at intervals starting 30 days after treatment, and each was autopsied aseptically. Liver, spleen, and kidney tissues were collected and stored at  $-20^{\circ}\text{C}$ . For virus isolation, the tissues from each bird were emulsified in buffered physiological saline (pH 7.2) and this was inoculated intracerebrally and intraperitoneally into 3 mice. At least two additional passages were performed if the responses to inoculation were negative. Diagnosis of psittacosis was based on the microscopic detection of Levinthan-Coles-Lillie (L.C.L.) inclusion bodies in brain tissue imprints from mice which showed symptoms of rough fur, meningismus, and incoordination usually within three to seven days following inoculation.

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TABLE I  
*Treatment of Psittacosis-Infected Flocks of Parrots  
with Chlortetracycline Hydrochloride in Sesame Oil\**

Flock number	Days after treatment	Virus isolation	
		No. positive/no. tested	%
I	69-79	0/21	0
	Untreated controls	12/24	50
II	25-78	0/42	0
	Untreated controls	4/10	40
III	18-52	0/30	0
	Untreated controls	11/20	55

\* Received 166 mg./Kg. body weight, intramuscularly, two injections two days apart.

The drug preparation induced some trauma in the inoculated muscles; however, the birds manifested little physical handicap from this. The injected area could be detected from one to three months later by fibrotic lesions and a yellowish deposit at the point of inoculation.

In three trials with naturally infected parrots, virus was recovered from 12 of 25, 4 of 10, and 11 of 20 of the untreated controls (table I). Psittacosis virus was not recovered from any of the 93 parrots that were treated with two injections of chlortetracycline in sesame oil. It appears that this procedure effectively cleared psittacosis virus from parrots.

It is likely that the host-parasite relationship in naturally infected birds may be different from that manifested by the acutely ill experimentally infected bird. Since this procedure is designed for public health application, naturally infected birds should yield more pertinent information. Fortunately, the infection rate among the untreated controls was high enough to give significant validity to the results with the treated birds.

Since psittacosis is considered an important zoonotic problem whose incidence in man can be equated with contact, eradication of the virus from birds should prevent the disease in man. This is an example of prophylaxis through chemotherapy.

#### BIBLIOGRAPHY

1. BEAUDETTE, F. R., ED.: *Psittacosis; Diagnosis, Epidemiology, and Control*, New Brunswick, N. J., Rutgers University Press, 1955.
2. WENNER, H. A.: Psittacosis-lymphogranuloma group of viruses, *Advances in Virus Research*, 5:39-93, 1958.
3. STEELE, J. H., AND SCRUGGS, J. H.: The Epidemiology of Psittacosis, in *Progress in Psittacosis Research and Control*, New Brunswick, N. J., Rutgers University Press, 1958; pp. 32-51.
4. WONG, S. C., AND COX, H. R.: Actions of Aureomycin against experimental rickettsial and viral infections, *Ann. New York Acad. Sc.* 51:290-305, 1948.
5. BRAINERD, H.; LENNETTE, E. H.; MIEKLEJOHN, G.; BRUYN, H. B., JR., AND CLARK, W. H.: The clinical evaluation of Aureomycin. *J. Clin. Investigation* 28:992, 1949.
6. WOODWARD, T. C.: Chloromycetin and Aureomycin: Therapeutic results, *Ann. Int. Med.* 31:53, 1949.
7. GREEN, T. W.: Aureomycin therapy of human psittacosis, *J.A.M.A.* 144:237, 1950.
8. VIDELA, C. A., AND PANDOLFO, J. P.: Neumonitis and virus en el adulto, *Rev. Asoc. méd. argent.* 64:511, 1950.
9. QUAN, S. F.; MEYER, K. F., AND EDDIE, B.: Attempts to cure parakeet psittacosis carriers with Aureomycin and penicillin, *J. Infect. Dis.* 86:132, 1950.
10. MEYER, K. F., AND EDDIE, B.: Chemotherapy of natural psittacosis and ornithosis. Field trial of tetracycline (polyotic hydrochloride), chlortetracycline, and oxytetracycline. *In: Antibiotics Annual 1954-1955*, New York, Medical Encyclopedia, Inc. 1955; pp. 544-555.
11. BUSSELL, R. H., AND POLLARD, M.: Treatment of psittacosis-infected parakeets with chlortetracycline. *In: Progress in Psittacosis Research and Control*, New Brunswick, N. J., Rutgers University Press, 1958; pp. 197-203.
12. POLLARD, M.: Therapy of psittacosis-infected parakeets with chlortetracycline, *Antib. & Chemo. In Press*.

# The Incidence of *Candida albicans* in Poultry. Evaluation of Nystatin and Chlorhydroxyquinoline in the Prevention of Experimental Moniliasis

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It is well established that overgrowth of *Candida albicans* in the crop can result in severe outbreaks of moniliasis (crop mycosis) in commercial poultry flocks.<sup>1,2</sup> Recent studies reported from this laboratory showed that nystatin,\* an antifungal antibiotic, was effective in the prevention and treatment of experimental moniliasis in chicks and turkeys.<sup>3,4</sup> In vitro experiments indicated that chlorhydroxyquinoline was active against *C. albicans* and other fungi.<sup>5</sup>

The economic losses caused by moniliasis in poultry have never been estimated. Limited surveys of the incidence of *C. albicans* in poultry were made in England.<sup>6,7</sup> However, there is very little published information on the incidence of this organism in poultry raised in the United States.

The purpose of this paper is to present data obtained in a preliminary study of the natural incidence of *C. albicans* in chickens and to report studies on the evaluation of nystatin and chlorhydroxyquinoline for the prevention of experimental moniliasis in chicks.

## MATERIALS AND METHODS

*Field Study.* The crop was removed from White Leghorn hens taken at random from the processing line at a New Jersey poultry processing plant. Using sterile cotton swabs, individual smears were made from each crop on Pagano-Levin agar medium.<sup>8</sup> The agar slants were incubated at room temperature for 72 hours and then examined for colonies showing the cream or pink color typical of *C. albicans* on this medium. A number of cultures, which were positive for this organism on the diagnostic medium, were further identified by growth characteristics and chlamydospore formation on corn meal agar.

*Laboratory Studies on Moniliasis.* Sexed White Plymouth Rock chicks were used. Feed and water was supplied ad libitum. The chicks were fed a purified ration composed of isolated soybean protein (Drackett "C-1"), cerelose, vegetable oil, and added minerals and vitamins. This ration was similar to that used by Edwards et al.<sup>9</sup>

Moniliasis was induced by oral inoculation with *C. albicans* Squibb strain no. 1539. The extent of infection was determined by rating the visible lesions of each excised crop using a scoring system ranging from 0 to 4.<sup>10</sup>

Nystatin and chlorhydroxyquinoline were added to the ration. Since chlorhydroxyquinoline sublimed from the ration, fresh feed mixes were made each week.

One mg. of the nystatin used in these experiments is equivalent to 2800 units.

## RESULTS AND DISCUSSION

*Field Study.* The data obtained in a field survey conducted in New Jersey are

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\* The trade name of E. R. Squibb & Sons Division, Olin Mathieson Chemical Corp., for nystatin is Mycostatin.

TABLE I  
*Field Surveys of the Incidence of Candida albicans in Poultry*

Investigator	Location	Number and kind of poultry examined	Number of crops in which <i>C. albicans</i> was found
Blaxland <sup>6</sup>	England	339 turkeys*	90 (26.5%)
Jordan <sup>7</sup>	England	240 chickens* over 6 months of age	60 (28.7%)
This study	United States (New Jersey)	84 normal hens	33 (39.3%)
		26 cull hens	7 (26.9%)
		110	40 (36.4%)

\* Data obtained from poultry that died from diseases other than moniliasis.

presented in table I along with similar data reported by Blaxland<sup>6</sup> and Jordan.<sup>7</sup> *C. albicans* was cultured from 36.4 per cent of the birds tested. Twenty-six of the hens were culls, which had been discarded from the processing line because of emaciation or the presence of infections. Gross examination of the crops showed no obvious mycotic lesions. A smooth, thickened membrane was present inside the crop wall in a large proportion of the hens. Further characterizations of the organisms from 20 of the crops that were positive for *C. albicans* on Pagano-Levin medium are presented in table II. All the isolates were yeasts with the exception of one gram-negative rod, which was neomycin resistant (the diagnostic medium contains a high level of this antibiotic). Eighteen of the 20 yeasts produced chlamydospores, thus confirming the results obtained using the Pagano-Levin medium.

These data are similar to those reported by Blaxland and Jordan in studies of poultry which had died from causes other than moniliasis and had been brought to a

TABLE II  
*Characterization of Yeasts from Chicken Crops on Pagano-Levin Medium and Chlamydospore Formation on Corn Meal Agar*

Sample	Colony color	Pagano-Levin medium			Corn meal agar. chlamydospores
		Colony growth characteristics	No. of colonies		
1*	Pink	Moist, raised	1		—
2*	Pink	Moist, raised	3		+
3*	Pink	Moist, raised	5		+
4a*	Rose center, cream rim	Moist, raised	3		+
4b*†	Red	Flat	20-30		+
5*	Rose center, pink rim	Moist, raised	15-20		+
6*	Rose center, pink rim	Moist, raised	4		+
7	Light pink	Moist, raised	10		+
8	Pink	Moist, raised	Numerous		+
9	Cream	Moist, raised	Numerous		+
10	Pink	Moist, raised	7		+
11	Light pink	Moist, raised	25-30		+
12	Cream	Moist, raised	Numerous		+
13	Cream	Moist, raised	Numerous		+
14	Rose center, pink rim	Moist, raised	15-20		+
15	Rose center, pink rim	Moist, raised	30-40		+
16	Cream	Moist, raised	Numerous		+
17	Light pink	Moist, raised	40-50		+
18	Rose center, cream rim	Moist, raised	6		+
19	Cream	Moist, raised	25-35		+
20	Pink	Moist, raised	15		—

\* Samples from cull chickens unsatisfactory for marketing.

† Gram-negative rod (neomycin resistant).

TABLE III  
*Nystatin for the Prevention of Moniliasis in Chicks*

Lot no.	Treatment*	Incidence of moniliasis, % (4 weeks)	Average crop score
331	None	0	0
332	<i>C. albicans</i> dose†	100	2.4
333	<i>C. albicans</i> plus 28 mg. nystatin per Kg. of ration	100	1.5
334	<i>C. albicans</i> plus 44 mg. nystatin per Kg. of ration	80	1.1
335	<i>C. albicans</i> plus 71 mg. nystatin per Kg. of ration	0	0
336	<i>C. albicans</i> plus 113 mg. nystatin per Kg. of ration	10	0.1

\* Ten chicks per treatment.

† One ml. oral dose given at 3 days of age; approximately  $1.9 \times 10^8$  cells per ml.

diagnostic laboratory for examination. The characterization of yeasts found by these investigators involved gross appearance of colonies, cell morphology, and carbohydrate fermentation reactions.

The presence of *C. albicans* in a high percentage of laying hens may account for the increased egg production observed by Carlson<sup>11</sup> in hens fed oxytetracycline and nystatin as compared with controls fed oxytetracycline alone.

*Laboratory Studies on Moniliasis.* The results of a typical experiment showing the effectiveness of nystatin in preventing moniliasis are presented in table III. In this experiment graded levels of nystatin were fed starting at 1 day of age, and at 3 days an oral dose of *C. albicans* was administered to some lots. Chicks were killed for crop examination at four weeks. The crop scores and incidence of moniliasis show that levels of nystatin ranging from 71 to 113 mg./Kg. of ration were effective in preventing development of this disease.

TABLE IV  
*Evaluation of Chlorhydroxyquinoline and Nystatin in the Prevention of Moniliasis in Chicks\**

Exper. no. Duration	Treatment, mg./Kg. of ration	No. of chicks started†	Avg. wt., Gm.	Incidence of moniliasis, %	Crop score	Mortality, %
I						
6 weeks	None	20	506 (5 wk.)	100 (5 & 6 wk.)	0.9 (18)†	5 (1-6 wk.)
	250 mg. chlorhydroxyquinoline	20	503 (5 wk.)	92 (5 & 6 wk.)	0.7 (16)	5 (1-6 wk.)
	2500 mg. chlorhydroxyquinoline	20	482 (5 wk.)	30 (5 & 6 wk.)	0.1 (10)	30 (1-6 wk.)
	105 mg. nystatin	20	540 (5 wk.)	53 (5 & 6 wk.)	0.4 (17)	5 (1-6 wk.)
	105 mg. nystatin	20	524 (5 wk.)	65 (5 & 6 wk.)	0.5 (20)	0 (1-6 wk.)
II						
5 weeks	None	15	305 (4 wk.)	100 (4 & 5 wk.)	0.9 (13)	0 (1-5 wk.)
	300 mg. chlorhydroxyquinoline	15	332 (4 wk.)	100 (4 & 5 wk.)	0.8 (14)	0 (1-5 wk.)
	900 mg. chlorhydroxyquinoline	15	333 (4 wk.)	100 (4 & 5 wk.)	0.9 (13)	0 (1-5 wk.)
	125 mg. nystatin	15	341 (4 wk.)	66 (4 & 5 wk.)	0.5 (12)	0 (1-5 wk.)
	125 mg. nystatin	15	365 (4 wk.)	25 (4 & 5 wk.)	0.5 (12)	0 (1-5 wk.)

\* All chicks infected with oral dose of *C. albicans*. Experiment I, 1 ml. dose (approx.  $1.64 \times 10^8$  cells) given at 5 days of age. Experiment II, 1 ml. dose (approx.  $2.2 \times 10^8$  cells) given at 7 days of age.

† Equal number of males and females used in experiment I. In experiment II, 10 females and 5 males used per treatment.

‡ Number of chicks autopsied.

The experimental plan and results of two tests involving chlorhydroxyquinoline and nystatin are shown in table IV. In experiment 1, a level of 250 mg. of chlorhydroxyquinoline per Kg. of ration was ineffective in preventing moniliasis in chicks. At 2500 mg./Kg. this compound showed a protective effect as indicated by low crop score and decreased incidence of infection; however, growth rate was depressed and mortality increased. In experiment 2, lower levels of chlorhydroxyquinoline (300 and 900 mg./Kg. of ration) were nontoxic but had no effect on the incidence or severity of moniliasis. All levels of this compound were corrosive to the galvanized feed troughs.

In both experiments, nystatin reduced the crop scores and the incidence of moniliasis.

The data reported herein on chlorhydroxyquinoline, along with the negative data reported by Blaxland et al<sup>12</sup> and by Underwood et al<sup>13</sup> on the use of copper sulfate, indicate that these compounds are ineffective in preventing moniliasis. Results obtained using nystatin show that it is the agent of choice for the prevention of moniliasis in poultry.

#### SUMMARY

Nystatin at levels ranging from 71 to 125 mg./Kg. of ration was effective in markedly reducing or preventing development of moniliasis in chicks orally infected with *C. albicans*.

Chlorhydroxyquinoline at levels of 250 to 900 mg./Kg. of ration was ineffective in chicks. A level of 2500 mg./Kg. showed a protective effect against moniliasis but was toxic.

A relatively high incidence of *C. albicans* was found in the crop of laying hens examined at a poultry processing plant.

#### BIBLIOGRAPHY

1. JUNGHERR, E. L.: Observations on a severe outbreak of mycosis in chicks, *J. Agric. Research* 46:169, 1933.
2. BUXTON, A.; DARCEL, C. LE Q.; GORDON, R. F., AND SMITH, C. H.: Suspected moniliasis in turkeys, *Vet. Rec.* 61:828, 1949.
3. YACOWITZ, H.; WIND, S.; JAMBOR, W. P.; SEMAR, R., AND PAGANO, J. F.: Use of Mycostatin<sup>R</sup> for the prevention of laboratory cases of moniliasis in chicks, *Poultry Sc.* 36:1171, 1957.
4. YACOWITZ, H., AND WIND, S.: Use of Mycostatin<sup>R</sup> for the prevention and treatment of moniliasis (crop mycosis) in chicks and turkeys, *Abst. 47th Annual Meeting Poultry Sc. A.*, 1958, p. 76.
5. PAGANO, J. F.: Personal communication, Squibb Institute for Medical Research, 1958.
6. BLAXLAND, J. D.: The causes of epidemic outbreaks of moniliasis in turkeys, *Proc. IX World's Poultry Congress*, 1952.
7. JORDAN, F. T. W.: The incidence of *Candida albicans* in the crops of fowls, *Brit. Vet. J.* 109:527, 1953.
8. PAGANO, J.; LEVIN, J. D., AND TREJO, W.: Diagnostic medium for differentiation of species of *Candida*. In: *Antibiotics Annual 1957-1958*, New York, Medical Encyclopedia, Inc., 1958, p. 137.
9. EDWARDS, H. M., JR.; CHIN, D.; NORRIS, L. C., AND HEUSER, G. F.: Differentiation of unidentified chick growth factors in liver, *J. Nutrition*, 57:329, 1955.
10. YACOWITZ, H.; WIND, S.; JAMBOR, W. P.; WILLETT, N., AND PAGANO, J. F.: Use of Mycostatin<sup>R</sup> for the prevention of moniliasis (crop mycosis) in chicks and turkeys. *Poultry Sc.* Submitted for publication.
11. CARLSON, C. W.: Some recent observations with dietary antibacterial and antifungal agents for laying hens. *Abst. 47th Annual Meeting Poultry Sc. Assoc.*, 1958, p. 13.
12. BLAXLAND, J. D., AND FINCHAM, I. H.: Mycosis of the crop (moniliasis) in poultry with particular reference to serious mortality occurring in young turkeys, *Brit. Vet. J.* 106:221, 1950.
13. UNDERWOOD, P. C.; COLLINS, J. H.; DURBIN, C. G.; HODGES, F. A., AND ZIMMERMAN, H. E., JR.: Critical tests with copper sulfate for experimental moniliasis (crop mycosis) of chickens and turkeys, *Poultry Sc.* 35:599, 1956.

# Studies of the Growth-Promoting Effect of Antibiotics in Chicks on a Purified Diet

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The rate of growth of young chicks under conventional conditions is usually increased if certain antibiotics are added to the diet.<sup>1-3</sup> This effect may be termed the "antibiotic growth response."<sup>4</sup> The response does not appear when the chicks are kept under "germ-free" conditions.<sup>5</sup> It may be concluded that the response is due to an effect on the intestinal bacteria that are normally present in chicks.

Various factors are known to affect the growth response of chicks to antibiotics. Coates and co-workers in 1951<sup>6</sup> observed that chicks did not respond to penicillin when they were housed in new and "uncontaminated" animal quarters, but that responses were obtained to penicillin when the chicks were reared in old "contaminated" quarters. Other workers, Hill et al<sup>7</sup> and Lillie et al,<sup>8</sup> presented similar data to indicate that the degree of contamination in the environment of chicks influenced their growth response to antibiotics.

There have also been reports on the effect of long-continued use of antibiotics in the same laboratory on the antibiotic growth response. Thus Libby and Schaible<sup>9</sup> reported a gradual reduction in the antibiotic response, which was, however, accompanied by a gradual increase in the weight of the control birds and a reduction in their mortality. The authors concluded that the continuous use of antibiotics over a long period produced a "lowered germ load or disease potential." Waibel et al<sup>10</sup> found that the average growth response in their laboratory of chicks to antibiotics was not so great in 1953 as it was between 1950 and 1952. However, they also found that average weight of the unsupplemented group was about 10 per cent higher in 1953 than it was during the previous period.

More recently, McGinnis et al<sup>11</sup> reported that the growth response of turkeys to procaine penicillin had been reduced after many years of routine use of this antibiotic in experimental diets. However, two other antibiotics, oleandomycin phosphate and erythromycin thiocyanate, gave a marked growth response comparable to that produced by penicillin in previous years. These authors suggested that the failure of antibiotics to stimulate growth may be due to the development of bacterial resistance to the commonly used antibiotics or to the shift toward nonsensitive bacterial strains.

In this communication, we report our observations on the growth response to penicillin in a purified diet over a period of several years during which the requirements for this antibiotic for maximum response increased.

## METHODS AND RESULTS

Day old crossbred chicks were placed in electrically heated batteries and given feed and water ad libitum. Procaine penicillin was used throughout. Two types of basal diets were used: Diet 1, containing casein, gelatin, and sucrose and supplemented with methionine and the known vitamins, has been previously described.<sup>12</sup> Diet 2 was a "practical type diet" containing 62 per cent corn, 20 per cent solvent-extracted soybean meal, 5 per cent corn gluten meal, 5 per cent fish meal, 2.5 per

TABLE I

*Effect of Diet on Growth Response of Chicks to Penicillin as Shown by Weights in Grams at 25 Days*

Supplement/Kg. of diet	Diet 1: casein sucrose, Gm.	Diet 2: natural ingredients, Gm.
None	243	323
100 mg. procaine penicillin	302	352
Per cent response over basal on procaine penicillin	24	9

cent distillers solubles, 2 per cent alfalfa meal, 1.5 per cent steamed bone meal, 0.5 per cent salt, and 2 per cent limestone containing magnesium sulfate, and supplemented with riboflavin, niacin, calcium pantothenate, and vitamins A and D. Diet 1 was used for the evaluation of antibiotics as growth promoting agents because this diet containing sucrose as the carbohydrate source had been found to give a better response with antibiotics than similar diets containing glucose or starch.<sup>12</sup> The substitution of glucose or starch for sucrose improved growth in the absence of penicillin, but growth on any of the three carbohydrates was the same when penicillin was added. A comparison of the responses to penicillin on diet 1 (casein-sucrose) and on diet 2 (practical type) is shown in table I. The response to 100 mg. of penicillin was 24 per cent on the sucrose diet and 9 per cent on the natural diet; however, the response to penicillin is variable as will be discussed later.

Occasional experiments were made using penicillin from 1951 to 1954. During this time the level of procaine penicillin required for maximum effect was about 1 mg. or less per Kg. of diet. During 1955, penicillin was used routinely as a control at a level of 10 mg./Kg. The growth response to this level of penicillin gradually decreased during 1955 but marked growth responses were obtained when the level was increased to 100 mg./Kg. A summary of the effectiveness of different levels of penicillin during the years from 1951 to 1958 is shown in table II.

A study was made of the variability of the weight at 25 days on the control and the antibiotic-fed chicks during 1957 and 1958. This is shown in figure 1 where the average weights of the control and supplemented birds in each experiment during a 20 months' period are given. These show that the growth of the control groups was somewhat more variable than the supplemented. The average weight of the controls for the 20 months' period was 244 Gm. with a coefficient of variability of 11.7. The average of the supplemented groups was 301 Gm. (23 per cent more)

TABLE II

*Effect of Long-continued Use on Growth Response to Penicillin*

Year	Per cent growth response over basal, mg. procaine penicillin/Kg. diet			
	1	2-5	10	100
1951	29(1)*	18(4)		
1952	21(7)	18(3)	26(1)	39(1)
1953	15(2)		12(1)	
1954	30(1)	20(1)	24(1)	
1955			10(15)	
1956			8(25)	15(4)
1957			6(9)	20(9)
1958			6(9)	24(16)

\* No. of observations per year.

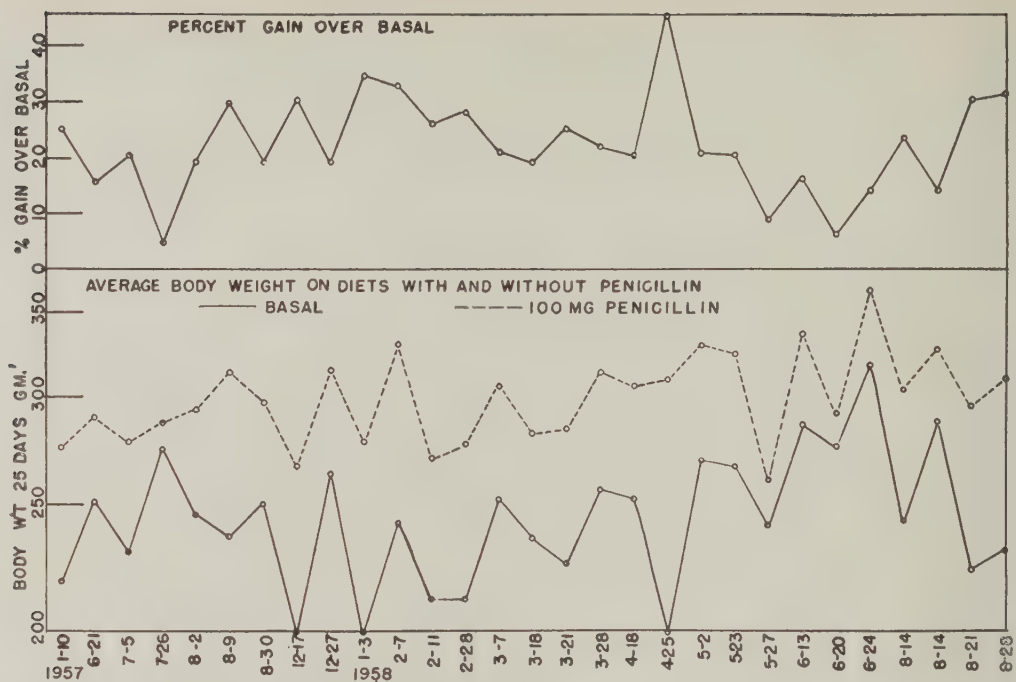


FIG. 1. Variations in the growth response of chicks to penicillin on a purified diet during 1957 to 1958.

TABLE III  
Growth Response of Chicks to Penicillin and Neomycin on Diet 1  
as Shown by Weights in Grams at 25 Days

Addition per Kg. of diet	1954*	1958†		
	Exper. 1	Exper. 2	Exper. 3	Exper. 4
None	209	212	247	253
1 mg. procaine penicillin	272			
5 mg. procaine penicillin	252			
100 mg. procaine penicillin		309	292	311
5 mg. neomycin sulfate	243			
10 mg. neomycin sulfate		307	259	266
30 mg. neomycin sulfate		302		

\* Triplicate groups of 12 chicks each.

† Duplicate groups of 12 chicks each.

TABLE IV  
Growth of Chicks on Diet 1 with Various Antibiotic Supplements, 1958,  
as Shown by Weights in Grams at 25 Days

Addition per Kg. of diet	Experiment				
	1	2	3	4	5
None	266	243	288	247	253
10 mg. procaine penicillin		297			258
30 mg. procaine penicillin		302			304
100 mg. procaine penicillin	292	304	326	292	311
2 mg. oleandomycin	266				
5 mg. oleandomycin	280		310		
10 mg. oleandomycin		322	339	287	274
10 mg. bacitracin			313	317	298
10 mg. streptomycin				213	

Oleandomycin was fed as the phosphate and streptomycin as the sulfate.

TABLE V

*Growth of Chicks in "Old" and "New" Environments on Diet 1 with Various Antibiotic Supplements*

Addition per Kg. of diet	Weights at			
	14 days		25 days	
	"Old"	"New"	"Old"	"New"
None	98	152	236	308
1 mg. procaine penicillin	113	149	265	316
10 mg. procaine penicillin	117	161	273	337
100 mg. procaine penicillin	134	152	307	329
20 mg. chlortetracycline hydrochloride	130	162	290	344

with a coefficient of variability of 8.0. Although the variability of the control birds was higher, the difference was not statistically significant.

A limited amount of data was obtained on a comparison of the effectiveness of neomycin in 1954 and in 1958. These results are given in table III and show that in 1954, 1 mg. of procaine penicillin gave the maximum response and that 5 mg. of neomycin sulfate gave an intermediate response. In 1958, 10 mg. of neomycin sulfate gave the same response as 100 mg. of procaine penicillin and then its activity declined.

Experiments with some other antibiotics in 1958 are summarized in table IV. The response to oleandomycin appeared to trend downward in successive experiments in which the antibiotic was fed at a level of 10 mg./Kg. of diet. Bacitracin produced a variable growth response at a level of 10 mg. but no response was obtained in a single experiment with streptomycin.

A comparison was made of the growth of chicks in two different environments. A single batch of chicks was used in this experiment. The "old" environment was an animal room that had been in constant use for 11 years. The "new" environment was a room in a nearby building in which chicks had not been previously kept and a cage was used that had been stored for six months following a thorough steam cleaning. The results are shown in table V. The growth response to the new environment as compared with the old environment was greater for the first two weeks than the response to the highest level of penicillin in the old environment. The findings serve to emphasize the concept that the antibiotic growth effect is of antibacterial origin rather than a direct effect on the animals.<sup>4,5</sup>

#### SUMMARY

A purified diet containing sucrose as the carbohydrate source was used for testing the growth-stimulating effects of antibiotics in chicks.

The amount of penicillin required to produce maximum growth response increased more than 10 times over a several year period of extensive use. However, penicillin retained its effect on the growth of chicks provided that higher levels were used. There was some indication of a downward trend in the growth-promoting activity of neomycin and oleandomycin during 1958. Unsupplemented chicks in a "new" environment grew as rapidly as chicks receiving penicillin in an "old" environment.

## BIBLIOGRAPHY

1. STOKSTAD, E. L. R., AND JUKES, T. H.: Further observations on the animal protein factor, *Proc. Soc. Exper. Biol. & Med.* 73:523, 1950.
2. STOKSTAD, E. L. R.: Antibiotics in animal nutrition, *Physiol. Rev.* 34:25, 1954.
3. JUKES, T. H., AND WILLIAMS, W. L.: Nutritional effects of antibiotics, *Pharm. Rev.* 5:381, 1953.
4. JUKES, T. H.: Antibiotics in Nutrition. Antibiotics Monograph, New York, Medical Encyclopedia, Inc., 1957.
5. FORBES, M., AND SUPPLEE, W. C.: Response of germ-free and conventionally reared turkey poults to dietary supplementation with penicillin and oleandomycin, *Proc. Soc. Exper. Biol. & Med.* 99:110, 1958.
6. COATES, M. E.; DICKINSON, C. D.; HARRISON, G. F.; KON, S. K.; PORTER, J. W. G.; CUMMINS, S. H., AND CUTHBERTSON, W. F. J.: A mode of action of antibiotics in chick nutrition, *J. Sc. Food Agric.* 3:43, 1952.
7. HILL, D. C.; BRANION, H. D.; SLINGER, S. J., AND ANDERSON, G. W.: Influence of environment on the growth response of chicks to penicillin, *Poultry Sc.* 32:462, 1953.
8. LILLIE, R. J.; SIZEMORE, J. R., AND BIRD, H. R.: Environment and stimulation of growth of chicks by antibiotics, *Poultry Sc.* 32:466, 1953.
9. LIBBY, D. A., AND SCHABBLE, P. J.: Observations on growth responses to antibiotics and arsenic acids in poultry feeds, *Science* 121:733, 1955.
10. WAIBEL, P. E.; ABBOTT, O. J.; BAUMANN, C. A., AND BIRD, H. R.: Disappearance of the growth response of chicks to antibiotics in an "old" environment, *Poultry Sc.* 33:1141, 1954.
11. MCGINNIS, J.; MERRILL, L. H.; FRY, R. E., AND JENSEN, L. S.: Use-history of antibiotics as related to their efficacy in promoting growth of turkeys, *Poultry Sc.* 37:810, 1958.
12. STOKSTAD, E. L. R.; JUKES, T. H., AND WILLIAMS, W. L.: The growth-promoting effect of Aureomycin on various types of diet, *Poultry Sc.* 32:1054, 1953.

# Studies on Monocontaminated Chickens (*Clostridium perfringens* or *Streptococcus faecalis*) Fed Penicillin

## Bacteriology, Growth, Serum Gamma Globulin, and Antibodies

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The normal microbial flora has been reported to have a stimulatory effect on certain morphological aspects of organs normally in close association with an abundant microbial population. Essentially, the intestine of chickens maintained in the complete absence of an intestinal flora (germ-free chickens) showed lower weight and lymphoid tissue content than the intestine of chickens reared under the usual laboratory conditions (conventional chickens).<sup>1-5</sup> Similarly, conventional chickens fed antibiotic incorporated in the diet at nutritional levels, displayed morphological characteristics different from those seen in nonantibiotic-treated conventional birds<sup>13-16</sup> but qualitatively similar to those seen under germ-free conditions.<sup>2,5</sup> Since both the complete absence of an intestinal flora and the presence of an antibiotic-affected flora result in underdevelopment of the intestinal lymphoid tissue, one may speculate that certain members of the normal intestinal flora have a stimulatory effect on the intestine, an effect that results in the "normal" picture, as it is observed in conventional life. Repression or modification of the presumably stimulatory strains of bacteria by feeding antibiotics results in a more or less "germ-free" picture.

Jukes<sup>6</sup> has summarized reports, prior to 1955, regarding the effects of antibiotics on various types of intestinal microorganisms. Generally, the results of different investigators, using various antibiotics in a variety of animal species, have not been consistent. Chickens fed penicillin generally show a decrease in clostridia<sup>7-10</sup> but not always so.<sup>11</sup> Lev et al<sup>12</sup> have reported the elimination of *Clostridium welchii* (syn. *Clostridium perfringens*) from the ceca of very young, penicillin-fed chicks but have also reported certain anomalous results, in which dietary penicillin caused no reduction in *Cl. welchii* numbers. In this latter case, a marked impairment in alpha toxin production by these bacteria was noted. In terms of a possible growth depressing action brought about by *Cl. welchii* toxins, impairment of toxigenicity would have the same effect on the host as elimination of the organism itself.

Along with the clostridia, the enterococci<sup>5,7-9,17</sup> and certain other intestinal organisms<sup>7,8</sup> are also reportedly decreased when penicillin is fed at low nutritional levels to conventional chickens. The possibility exists that the reduction, elimination, or physiological alteration of intestinal microorganisms by dietary penicillin may be responsible for the decreased stimulation of organs with which they are normally in contact. A similar cause and effect relationship may exist by which antibiotics may relieve microbial growth depressing action on the host. However, the alleviation of growth depression and the lower tissue stimulation observed with dietary antibiotics do not always go hand in hand,<sup>5,16</sup> nor are the microbiological changes always consistent. The use of gnotobiotic techniques, by which germ-free animals may be observed per se or inoculated with known organisms, offers an op-

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portunity to study the effect of individual or selectively combined members of the normal intestinal flora on the host. Antibiotics may be brought in as an additional variable. However, it is important to recognize that this type of experimentation is subject to certain restrictions imposed by the selected conditions employed, both from the standpoint of the host and the microorganism(s).

*Cl. perfringens* was chosen as a monocontaminant in one series of experiments because of its potential as a possible growth depressing and tissue stimulating agent and because of its sensitivity to penicillin. *Cl. perfringens* produces a battery of hemolytic and necrotizing toxins, capable of attacking physiological tissue substrates, such as collagen, hyaluronic acid, lecithin, and desoxyribonucleic acid. *Streptococcus faecalis* was selected as a monocontaminant in another experiment, because of its reported reduction in numbers during dietary penicillin administration.

Observations have been made on the bacteriology, growth, serum gamma globulin, and antibody titers of chickens reared under selected monocontaminated conditions with and without administration of dietary penicillin. The paper by Gordon and Bruckner-Kardoss<sup>24</sup> refers to the size and cellularity of the ileocecal lymph nodes and to the distribution of reticulo-endothelial elements in the intestinal wall of these same chicks. Data on conventional animals with and without dietary penicillin are also included.

#### METHODS

Germ-free white Leghorn chickens were obtained by methods essentially similar to those described by Reyniers et al.<sup>1</sup> At the completion of the hatch, sealed ampoules, containing viable pure cultures of *Cl. perfringens* or *Str. faecalis* were passed into the isolation unit via a 2 per cent mercuric chloride germicidal trap. The chicks were inoculated orally with a cotton swab dipped into the culture. The germ-free status of the chickens prior to inoculation was determined by previously described methods.<sup>18</sup> The purity of the monocontaminated state was checked periodically during the course of the runs.

The chicks were divided into two groups within the same germ-free unit<sup>1</sup> and fed steam-sterilized, practical diet L-289F, with or without radiation sterilized, procaine penicillin G at 50 mg./Kg. diet, respectively.<sup>5</sup> The chicks were weighed at weekly intervals until sacrificed at four and one half or nine weeks.

Animals were taken individually from the monocontaminated environment and sacrificed within 10 minutes after removal to the laboratory. Sacrifice was by exsanguination following electronarcosis. The blood was allowed to clot at room temperature and the serum separated after overnight refrigeration. Sera were stored frozen until they could be evaluated for gamma globulin<sup>19</sup> and agglutinin titer<sup>20</sup> responses to monocontamination.

Samples for bacterial counts were taken from the contents of the cecum and lower ileum (approximately 0.05 to 0.25 Gm. of contents made up to 10 ml. in sterile water) and plated out quantitatively. Counts from *Str. faecalis* monocontaminated chicks were made in aerobic tryptone glucose extract agar plates (Difco<sup>21</sup>) incubated two days at 37 C. Counts from *Cl. perfringens* monocontaminated birds were made on anaerobic Bray dishes containing anaerobic agar (BBL<sup>22</sup>) and incubated two days at 37 C. Penase (Difco) was added (1 ml./100 ml. medium) to inactivate any residual penicillin activity in the intestinal contents. All counts were corrected to numbers of bacteria/Gm. dry weight of intestinal contents.

TABLE I

*Bacteriology of the Lower Ileum in Conventional Chickens Fed With or Without Procaine Penicillin G, 50 mg./Kg. Diet*

Microbial grouping	Logarithm of numbers of bacteria/Gm. dry ileum contents							
	Diet plus penicillin				Diet only			
	No.	Mean	Min.*	Max.*	No.	Mean	Min.*	Max.*
Total count (aerobic)	3	7.71	6.88	8.03	3	7.74	7.01	7.98
Total count (anaerobic)	3	7.91	7.02	8.07	3	7.71	7.01	8.02
Lactobacilli	3	7.91	6.76	8.10	3	7.69	6.98	7.88
Streptococci	3	6.35	6.26	6.48	3	7.64	6.80	7.92
Micrococci	3	3.83	3.62	3.99	3	4.48	3.13	4.78
Coliform bacteria	3	6.40	5.88	6.59	3	6.19	5.85	6.51
Clostridia	3	2.52	<1.0†	2.76	3	5.63	4.50	5.99
Molds	3	<1.0	—	—	3	<1.0	—	—
Yeasts	3	4.42	3.66	4.74	3	2.63	<1.0	3.10

\* Min. and max. = minimum and maximum numbers of bacteria observed, respectively.

† Organisms not recovered from the most concentrated sample preparations employed are arbitrarily recorded as <1.0.

Differential bacterial counts were made from the intestinal contents of conventional and antibiotic-fed conventional chickens on the following media: total count (aerobic), Tryptone glucose extract agar (Difco); total count (anaerobic), Tryptone glucose extract agar (Difco); lactobacilli, LBS medium (BBL); streptococci, Azide blood agar base (Difco); coliform bacteria, Violet red bile agar (Difco); micrococci, *Staphylococcus* medium 110 (Difco); yeasts, Potato dextrose agar (Difco); molds, Potato dextrose agar (Difco); and clostridia, modified sulfite-iron agar.<sup>23</sup>

Conventional white Leghorn chickens used in these studies were hatched in a commercial-type incubator and maintained in the animal room in wire brooders. They were fed the same sterilized diet and penicillin used in the monocontaminated runs.

## RESULTS

**Bacteriology.** Bacteriological findings in the ileum contents of conventional chickens fed diet L-289F, with or without added procaine penicillin G, are shown in table I. While only 3 animals/group were observed, penicillin tended to decrease the clostridia to virtual elimination and also seemed to cause a slight reduction in streptococci. The latter trend was also reported in cecal contents of chicks.<sup>7</sup> These findings were a basis for selecting the bacteria to be used in the monocontaminated experiments.

TABLE II

*Bacterial Count in 32 Day Old Chickens Monocontaminated with Clostridium perfringens and Fed With and Without Penicillin*

Group	Logarithm of numbers/Gm. dry contents			
	Ileum		Cecum	
	No.	Mean (min.—max.)*	No.	Mean (min.—max.)*
Diet only	4	7.85 (6.66 — 8.20)	4	8.88 (8.40 — 9.19)
Diet plus penicillin	4	2.00 (<1.0† — 2.60)	4	2.34 (<1.0 — 2.94)

\* Min. — max. = minimum and maximum numbers of bacteria observed.

† Organisms not recovered from the most concentrated sample preparations employed are arbitrarily recorded as <1.0.

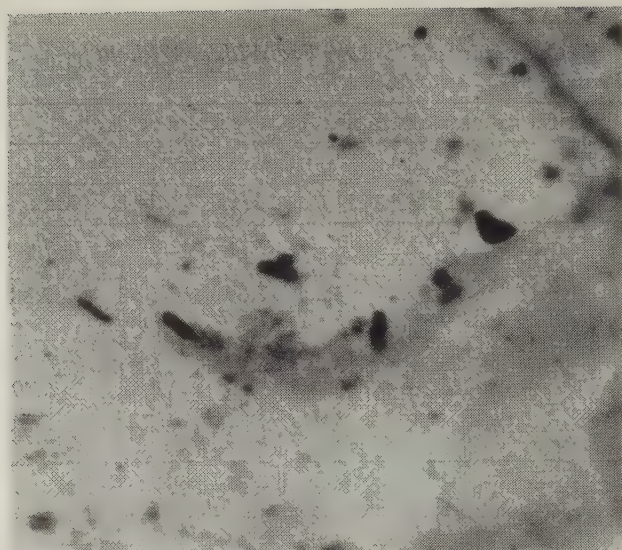


FIG. 1. (Top) Cecal contents from a chicken monocontaminated with *Clostridium perfringens* and fed procaine penicillin G at 50 mg./Kg. diet.

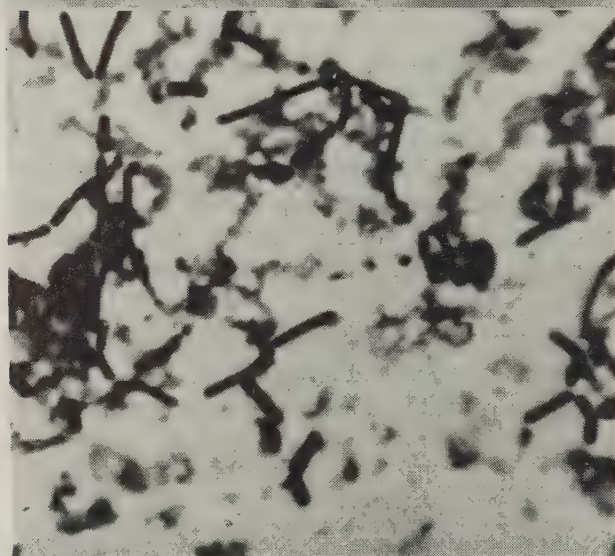


FIG. 2. (Bottom) Cecal contents from a chicken monocontaminated with *Clostridium perfringens* and fed diet without added penicillin.

The antibacterial effect of feeding penicillin to chickens monocontaminated with *Cl. perfringens* is shown in table II. Penicillin virtually eliminated *Cl. perfringens* from the intestinal tract of these animals. No clostridia were recovered from three of the four penicillin-fed animals examined (see discussion). The chickens fed only the basal diet harbored a substantial *Cl. perfringens* population. Figures 1 and 2 show photomicrographs of cecal contents from penicillin-treated and nontreated chicks housed in the same *Cl. perfringens* monocontaminated isolation unit. They illustrate the degree of inhibition by dietary penicillin for this organism in the intestine. The ileum presented a similar picture.

Table III presents the number of *Str. faecalis* in the ileum and cecum contents of chickens monocontaminated with this organism and fed with or without penicillin in the diet. In penicillin-treated chicks sacrificed at 32 days, a more than three hundredfold decrease in average bacterial count was found in the ileum, when compared to the nontreated group. However, this difference was not seen in the cecum at 32 days, nor was any difference noted in the ileum or cecum in chicks sacrificed at 62 days. Despite the decrease seen in the ileum of the 32 day old

TABLE III

*Bacterial Count in Chickens Monocontaminated with Streptococcus faecalis and Fed With and Without Penicillin*

Group	Logarithm of numbers/Gm. dry contents			
	Ileum		Cecum	
	No.	Mean (min.—max.)*	No.	Mean (min.—max.)*
32 day old chicks				
Diet only	4	9.77 (9.39 — 10.07)	4	10.76 (9.98 — 10.96)
Diet plus penicillin	4	7.25 (6.47 — 7.66)	4	10.64 (10.47 — 10.69)
62 day old chicks				
Diet only	3	10.38 (9.35 — 10.64)	4	10.87 (10.73 — 11.05)
Diet plus penicillin	4	9.88 (7.52 — 10.10)	4	10.56 (10.24 — 10.76)

\* Min.—max. = minimum and maximum numbers of bacteria observed.

penicillin-fed chicks, a substantial population of streptococci was still maintained in these birds.

*Growth.* Observations on the effect of dietary penicillin on the growth of *Cl. perfringens* monocontaminated chicks are shown in table IV for two experiments involving small numbers of animals. The data are presented for the individual experiments and also pooled. Penicillin gave no antibiotic growth effect in the males. In females of the first experiment, a possible growth effect was indicated. However, a growth effect was not evident in the second experiment nor in the pooled data.

In the experiment depicted in table V, penicillin appeared to produce an early growth effect in males monocontaminated with *Str. faecalis*. The effect approached statistical significance during the first three weeks of life but seemed to disappear by the fourth week. Data are also given for females, but numbers were too few for comment.

*Gamma Globulin and Agglutinin Titers.* Results of gamma globulin levels and agglutinin titers in *Str. faecalis* monocontaminated chickens are shown in table VI. Previous publications on electrophoretic studies of serum proteins in germ-free and

TABLE IV

*Observations on the Effect of Dietary Penicillin on the Growth of Chickens Monocontaminated with Clostridium perfringens*

Exper. no.	Penicillin fed	Sex	No. of animals	Weight, Gm., at ages, wk.			
				1	2	3	4
I	+	♂	5	63±3*	125±8	209±12	273±15
	—	♂	3	61±4	123±8	209±14	290±17
	+	♀	3	70±5	124±2	194±5	239±7
	—	♀	4	56±5	106±5	163±10	210±15
II	+	♂	3	64±5	117±6	180±9	271±11
	—	♂	4	69±3	122±4	177±11	261±18
	+	♀	4	65±4	113±8	174±10	252±20
	—	♀	3	69±3	114±2	167±8	244±13
I + II	+	♂	8	63±4	122±9	198±18	272±14
	—	♂	7	66±5	122±6	190±20	274±23
	+	♀	7	67±4	118±9	182±13	246±17
	—	♀	7	61±8	110±6	165±10	225±22

\* Arithmetic mean ± standard deviation of series.

TABLE V

Observations on the Effect of Dietary Penicillin on the Growth of Chickens  
Monocontaminated with *Streptococcus faecalis*

Group	Sex	No. of animals	Weight, Gm., at ages, wk.					No. of animals	9
			1	2	3	4			
Diet plus penicillin	♂	7	67±6†	122±13	205±22	263±29		3	829±84
Diet only	♂	6	57±6	105±6	180±7	242±22		3	801±28
Diet plus penicillin	♀	2	63 { 69 56	112 { 120 104	191 { 202 180	245 { 251 238		2	744 { 738 750
Diet only	♀	3	61 { 65 60 58	113 { 118 115 106	194 { 205 202 175	251 { 266 269 218		2	755 { — 765 745

† Arithmetic mean ± standard deviation of series.

conventional chickens<sup>4, 19</sup> have shown germ-free chickens to have less serum gamma globulin than their conventional counterparts. Monocontamination of newly hatched, germ-free chickens with a pure culture of *Str. faecalis* for 62 days resulted in bringing the gamma globulin level up to at least that seen in conventional chicks. Feeding procaine penicillin had no influence on the levels reached at that age.

Agglutinin titers against *Str. faecalis* were demonstrated in both penicillin-treated and nontreated chicks monocontaminated with the homologous organism. Two conventional chicks of comparable age gave titers at the lowest range observed in the monocontaminated group. Germ-free chicken sera showed low gamma globulin levels and no *Str. faecalis* agglutinins.

## DISCUSSION

In the introductory remarks, reference was made to the changes brought about by dietary penicillin in the intestinal flora of conventional chickens. The limited data presented in table I confirmed the decrease in intestinal clostridia and enterococci, often reported in the literature. Since penicillin-fed conventional chickens (as well as germ-free chicks) also show lower weight and lymphoid tissue content in organs normally associated with the microbial flora (e.g., the intestines), it is a reasonable

TABLE VI

Serum Gamma Globulin Content and *Streptococcus faecalis* Agglutinin Titers in  
Germ-free, Conventional, and Monocontaminated Chickens

Chicken group status	Age, days	No. of animals	Gamma globulin*		Anti-agglutinin titers		
			Per cent	mg. per cent	No. of animals	Geometric mean	Range
<i>Str. faecalis</i> plus penicillin†	62	4	18.9±3.9	757±218	5	1/49	1/16-1/128
<i>Str. faecalis</i> no penicillin	62	4	21.2±4.1	760±141	5	1/85	1/32-1/128
Conventional	68	—	—	—	2	1/16	1/16
Conventional	56	5	15.9±1.8 <sup>4</sup>	—	—	—	—
Conventional	75	11	21 <sup>19</sup>	800±55 <sup>19</sup>	—	—	—
Germ-free	56	4	12.5±2.7 <sup>4</sup>	—	—	—	—
Germ-free	75	5	9 <sup>19</sup>	321±43 <sup>19</sup>	5	0	0

\* Data on gamma globulin marked with reference numbers taken from previous publications and presented for comparison.

† Monocontaminant and penicillin treatment indicated.

speculation that a cause and effect relationship may exist between these bacteria and the morphological characteristics of the host's intestine. The findings reported herein and discussed in the following contribute to the validity of this speculation.

Chickens monocontaminated with pure cultures of *Cl. perfringens* or *Str. faecalis* maintained an abundant population of these organisms in the intestinal tract. Gordon and Bruckner-Kardoss,<sup>24</sup> comparing monocontaminated versus germ-free chicks, have found that monocontamination with *Cl. perfringens* brought about an increase in the number of reticulo-endothelial cells in the wall of the intestine. The size and cellularity of the ileocecal lymph nodes were also increased, and the values approached those seen under conventional conditions. To a lesser extent, this was also true for *Str. faecalis*. The feeding of penicillin to the monocontaminated birds tended to preserve the germ-free-like characteristics.

From the bacteriological standpoint, dietary penicillin at 50 mg./Kg. diet drastically reduced the intestinal *Cl. perfringens* population of monocontaminated chicks to virtual elimination of the organism. In six of eight counts from 1 ml. samples of ileum or cecum content suspensions no colonies developed. However, very small numbers of viable bacteria were still present in the feces of penicillin-treated chicks, since undiluted feces inoculated into fluid thioglycollate medium<sup>22</sup> resulted in growth and recovery of the organism.

Penicillin feeding decreased the number of streptococci in the ileum but not in the cecum of 32 day old *Str. faecalis* monocontaminated chicks. However, the decrease was mild in comparison to *Cl. perfringens*, and a substantial residual population of *Str. faecalis* was maintained in the intestinal tract during penicillin treatment. The bacterial count in the 62 day old *Str. faecalis* group was not influenced by dietary penicillin.

*Str. faecalis* stimulated gamma globulin and homologous agglutinin production above the respective low and absent germ-free levels (table VI). The failure of penicillin to bring about a drastic reduction in numbers of *Str. faecalis* may also explain the apparent failure of the antibiotic to cause any appreciable lowering of the gamma globulin and agglutinin levels in the same birds. Unfortunately, serum gamma globulin and agglutinin titers are not yet available for the *Cl. perfringens* group. It will be interesting to observe whether *Cl. perfringens* as a monocontaminant can bring about a higher than germ-free gamma globulin level and also stimulate the production of homologous agglutinins, which can be "reversed" by penicillin. Indications that this might occur are: (1) Reticulo-endothelial cells generally associated with antibody formation (plasma cells, lymphocytes) are increased over low germ-free levels in the intestinal wall of *Cl. perfringens* monocontaminated chicks but remain at low levels when dietary penicillin is fed;<sup>24</sup> (2) size and cellularity of the ileocecal lymph nodes are increased over germ-free levels in the monocontaminated chicks but are reduced by penicillin feeding;<sup>24</sup> and (3) the numbers of intestinal *Cl. perfringens* are drastically reduced by penicillin, thus limiting the original stimulus. However, the observations in (1) and (2) only represent a small portion of the antibody producing areas in the body. Much may also depend on the relative antigenicity of *Cl. perfringens* as to whether differences in gamma globulin and agglutinin levels between penicillin-treated and nontreated chicks would be discernible.

Conclusions cannot be drawn from the growth data presented, due to the small number of chickens observed. The great variation in body weights of chickens reared under seemingly identical conditions makes larger numbers of animals necessary. At the same time, limited germ-free cage space and the necessity of subdivid-

ing groups by sexes kept the number of available animals small per experiment. A transient antibiotic growth effect was suggested in male *Str. faecalis* monocontaminated chicks. A growth effect was also suggested in females of one out of two experiments involving *Cl. perfringens* monocontamination. More work is needed along these lines. Recently, Forbes et al<sup>25</sup> have reported that *Cl. welchii*, as the sole component of the intestinal flora, depressed the growth of chicks, when compared to the growth of germ-free chickens, and that penicillin relieved the growth depression. They were unable to produce this same effect with several other microorganisms, singly or in combination.

#### SUMMARY

Penicillin has been observed to produce the following effects when fed at nutritional levels to chickens: (1) virtually eliminated *Clostridium perfringens* from the ileum of conventional and *Cl. perfringens* monocontaminated chickens; (2) caused slight decrease in streptococci of the ileum in 32 day old conventional and *Str. faecalis* monocontaminated chickens; and (3) tended at times to show an antibiotic growth effect in monocontaminated chickens. However, the small number of animals observed and inconsistencies in the effect of sex indicate more work is needed along these lines.

Monocontamination with *Str. faecalis* brought about a rise in the low germ-free gamma globulin level, which reached the level observed in conventional chickens. Monocontamination also stimulated production of homologous agglutinins, which were absent in germ-free chicks. Penicillin had no effect on these levels under monocontaminated conditions. The possibilities of observing a penicillin effect in *Cl. perfringens* monocontaminated chickens are discussed.

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#### BIBLIOGRAPHY

1. REYNIERS, J. A.; TREXLER, P. C.; ERVIN, R. F.; WAGNER, M.; LUCKEY, T. D., AND GORDON, H. A.: Rearing Germ-free Chickens, Lobund Reports no. 2, Notre Dame, Ind., University Press, 1949, pp. 1-116.
2. GORDON, H. A.: A colloquium. Studies on the growth effect of antibiotics in germfree animals. A morphological and biochemical approach, Lobund Institute, University of Notre Dame, Notre Dame, Indiana, 1952.
3. GORDON, H. A.: Morphologic characterization of germfree life, Bull. New York Acad. Med. 31:236-239, 1955.
4. THORBECKE, G. J.; GORDON, H. A.; WOSTMANN, B. S.; WAGNER, M., AND REYNIERS, J. A.: Lymphoid tissue and serum gamma globulin in young germfree chickens, J. Infect. Dis. 101:237-251, 1957.
5. GORDON, H. A.; WAGNER, M., AND WOSTMANN, B. S.: Studies on conventional and germ-free chickens treated orally with antibiotics. In: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, pp. 248-255.
6. JUKES, T. H.: Antibiotics in Nutrition, New York, Medical Encyclopedia, Inc., 1955.
7. ANDERSON, G. W.; SLINGER, S. J., AND PEPPER, W. F.: Effect of dietary organisms on the growth and cecal flora of chicks. Poultry Sc. 31:905, 1952.
8. SLINGER, S. J.; HAUSER, M. M., AND PEPPER, W. F.: The correlation between feed consumption and fecal flora in chicks, J. Nutrition 52:75-88, 1954.

9. ELAM, J. F.; JACOBS, R. L.; FOWLER, J., AND COUCH, J. R.: Effect of dietary clostridia upon growth-promoting responses of penicillin, *Proc. Soc. Exper. Biol. & Med.* 85:645-648, 1954.
10. ELAM, J. F.; JACOBS, R. L.; TIDWELL, W. L., AND COUCH, J. R.: Possible mechanisms involved in growth promoting responses obtained from antibiotics, *Poultry Sc.* 31:914, 1952.
11. SMYSER, C. F., JR.; CLEVERDON, R. C.; KULP, W. L., AND MATTERSON, L. D.: Effect of dietary antibiotics on number of *Clostridium perfringens* in feces of chickens, *Antib. & Chemo.* 2:363-365, 1952.
12. LEV, M.; BRIGGS, C. A. E., AND COATES, M. E.: The gut flora of the chick: 3. Differences in cecal flora between 'infected,' 'uninfected' and penicillin-fed chicks. *Brit. J. Nutrition* 11:364-372, 1957.
13. PEPPER, W. F.; SLINGER, S. J., AND MOTZOK, I.: Effect of Aureomycin on the niacin and manganese requirements of chicks, *Poultry Sc.* 32:656-660, 1953.
14. COATES, M. E.; DAVIES, M. K., AND KON, S. K.: The effect of antibiotics on the intestine of the chick, *Brit. J. Nutrition* 9:110-119, 1955.
15. JUKES, H. G.; HILL, D. C., AND BRANION, H. D.: Effect of feeding antibiotics on the intestinal tract of the chick, *Poultry Sc.* 35:716-723, 1956.
16. HILL, C. H.; KEELING, A. D., AND KELLY, J. W.: Studies of the effect of antibiotics on the intestinal weights of chicks, *J. Nutrition* 62:255-267, 1957.
17. DIXON, J., AND THAYER, R. H.: Observations on the growth-promoting action of antibiotics in the chick, *Poultry Sc.* 30:910, 1951.
18. WAGNER, M.: Determination of germfree status, *Ann. New York Acad. Sc.* To be published.
19. WOSTMANN, B. S., AND GORDON, H. A.: Electrophoretic studies on serum proteins of young germfree, conventional and antibiotic treated conventional chickens, *Proc. Soc. Exper. Biol. & Med.* 97:832-835, 1958.
20. REYNIERS, J. A.; WAGNER, M.; LUCKEY, T. D., AND GORDON, H. A.: Survey of germfree life. I. The white Wyandotte bantam and white Leghorn chicken. (Morphological, biochemical, serological and nutritional characteristics), *Lobund Reports* no. 3, Notre Dame, Ind., University Press. To be published.
21. ANON.: Difco Manual, 9th ed. Difco Laboratories Inc. Detroit, Michigan.
22. ANON.: BBL-Products for the Microbiological Laboratory, 4th ed. Baltimore Biological Laboratory, Inc. Baltimore, Md.
23. MOSSEL, D. A. A.; DEBRUIN, A. S.; VAN DIEPEN, H. M. J.; VENDRIG, C. M. A., AND ZOUTEWELLE, G.: The enumeration of anaerobic bacteria, and of *Clostridium* species in particular, in foods, *J. Appl. Bact.* 19:142, 1956.
24. GORDON, H. A., AND BRUCKNER-KARDOSS, E.: The distribution of reticulo-endothelial elements in the intestinal mucosa and submucosa of germ-free, monocontaminated, and conventional chickens orally treated with penicillin. *In: Antibiotics Annual 1958-1959*, New York, Medical Encyclopedia, Inc., 1959, p. 1012.
25. FORBES, M.; PARK, J. T., AND LEV, M.: The roll of the intestinal flora in the growth response of chicks to dietary penicillin, presented at the Conference on Germfree Vertebrates, New York Academy of Sciences, 1958. To be published.

# The Distribution of Reticulo-endothelial Elements in the Intestinal Mucosa and Submucosa of Germ-free, Monocontaminated and Conventional Chickens Orally Treated with Penicillin

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The abundant supply of reticulo-endothelial (RE) cells in the wall of the normal intestinal canal has for many years been thought of as a host response to the presence of the normal microbial flora in the intestinal lumen.<sup>1,2</sup> More recently, this thesis found experimental support in the observations that were made in germ-free animals, i.e., in hosts whose intestinal tract did not harbor a viable flora. In general, a considerable deficit of lymphoid elements has been described in the intestinal wall and its associated lymph nodes of various germ-free species.<sup>3-10</sup> In addition, low numbers of free cells (rat)<sup>4</sup> and the practical absence of plasma cells (chicken)<sup>10</sup> have been recorded in the cecal wall under germ-free conditions. Recently, on establishment of the normal flora in rats with a germ-free history, considerable increase of lymphoid tissue has been found in the gastrointestinal tract.<sup>11</sup>

Another type of evidence that supports the thesis of the reticulo-endothelial cell stimulation by the normal intestinal flora originates from experiments in which antibacterial agents are fed to the normal (conventional) host. In addition to clinical observations, which are usually made in this field with therapeutic dosage of antibiotics,<sup>12</sup> it has been found that administration of these agents at low nutritional levels to conventional animals will result in a deficit of the intestinal lymphoid tissue, which is qualitatively comparable to the underdevelopment of this tissue in the germ-free host.<sup>13,14</sup>

Thus, the presently available information on the reticulo-endothelial cell versus contaminant relationship, particularly as studied with germ-free-type techniques, refers primarily to the nodular lymphoid tissue. The disseminated reticulo-endothelial cells, on the other hand, which are less amenable to quantitative observation, have been relatively neglected. Yet information in this field is of patent importance, as the function of these cells is known to be closely associated with the defenses of the body.

In accordance with these, the aim of the present work was to study the quantitative occurrence of scattered reticulo-endothelial cells in the intestines of germ-free and conventional chickens, including also such animals that live in association with single species of bacteria of the normal flora. The primary purpose of this phase was to search for characteristics that germ-free life and the bacterial flora impart to the animal host. In a successive step, the effect of penicillin was studied on the reticulo-endothelial cell count of the same animal groups in order to contribute to the knowledge of the action mechanism of this drug on the host and its contaminants.

## METHODS

White Leghorn chickens were hatched and maintained under germ-free, mono-

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contaminated (with *Clostridium perfringens* or *Streptococcus faecalis*), and conventional conditions.<sup>15,16</sup> The sterilized, fortified broiler ration and antibiotic fed to the animals (L289F, 50 mg. procaine penicillin G per Kg. diet) was the same as previously reported.<sup>14</sup> The age of the animals was 30 to 35 and 60 to 68 days. The sexes were mixed. Prior to sacrifice (in the morning hours) the animals were starved overnight. An exception from this rule was made in the case of the monocontaminated birds, which were not starved in order to ensure an adequate amount of intestinal contents for bacteriological studies. The animals were sacrificed in the open laboratory environment by exsanguination in electronarcosis, a maximum of 10 minutes after their removal from the original housing units. Immediately after sacrifice, approximately 10 ml. of the fixative used (Bouin) was injected into the pleuroperitoneal cavity. As it was endeavored to study a segment of the intestine that is normally rich in microbial flora, a portion of the lower ileum was excised that was within 10 mm. of the ileocecal-colic junction. Fixation time was approximately 12 hours in the refrigerator. The tissues were embedded in celloidin-paraffin, according to the method of Apáthy.<sup>17</sup> Uniform 4 $\mu$  sections of the intestine were prepared and stained according to Maximow's hematoxylin-eosin-azur II technique.<sup>18</sup>

The count of the disseminated cells was made as follows: The complete cross-sections of the intestine (cut to 90 degrees to the longitudinal axis as closely as possible) were projected, and the outline of the epithelial tissue, submucosa, and muscularis were traced on paper using 600x linear magnification. In a successive step of the procedure, a distinct area of the previously mentioned tissues was measured with a planimeter. In these areas (each approximately 0.4 sq. mm.), all listed cells were counted under oil immersion. The results were expressed in cells/sq. mm. tissue. Three separate counts were made in three different sections originating from 1 animal. The arithmetic mean of these three values was taken as a basis for the calculation given in the tables, and when it appeared permissible, for the establishment of standard deviation of mean (thus under "no." in the tables, actually one third of the number of counts is listed). "Epithelial tissue" refers to the lining of the villi proper together with the epithelium of the Lieberkühn krypts, "subepithelial tissue" to all nonepithelial elements of the mucosa and submucosa. RE cells are relatively rare in the muscularis, and little if any differences were found in this respect among the various experimental groups; thus these values are not reported. As there was no appreciable effect of age and sex on the RE counts of this series, the results of the two age groups were pooled (except the *Cl. perfringens* group, which consisted only of 30 to 35 day old animals). The weight and cellularity of the cecal lymph nodes were determined in the same way as previously reported.<sup>14</sup>

## RESULTS

The total and differential counts of the scattered RE cells in the lower ileum of the chicken are given in tables I and II for various experimental groups.\* On bacterial contamination and antibiotic treatment, the host's response was indicated primarily in the count of certain cell types in a given tissue compartment. These were the Schollen leukocytes in the epithelium and the lymphocytes and plasma cells

\* Throughout this paper the symbol "gf" is used for germ-free, "strep" for *Str. faecalis*, "clostr" for *C. perfringens*, "conv" for conventionally contaminated chickens; - stands for untreated, + for penicillin-treated chickens.

TABLE I

*Total Count of Scattered Reticulo-endothelial Cells in the Mucosa and Submucosa of the Lower Ileum in Germ-free, Monocontaminated, and Conventional White Leghorn Chickens Treated and Untreated with Procaine Penicillin G (50 mg./Kg. Diet). (Number of Cells Is Given Per Square mm. of Tissue in 4  $\mu$  Sections; age: 30 to 68 Days; both Sexes)*

	No.	Epithelial tissue		Subepithelial tissue		Total	
		Mean	Standard deviation of mean	Mean	Standard deviation of mean	Mean	Standard deviation of mean
gf <sup>-</sup>	11	360	93	570	160	930	200
gf <sup>+</sup>	6	250	42	530	190	770	220
strep <sup>-</sup>	6	380	210	1080	260	1460	370
strep <sup>+</sup>	6	380	138	840	180	1220	300
clostr <sup>-*</sup>	3	530	22	1470	300	2000	280
clostr <sup>+</sup> *	3	260	42	920	210	1180	240
conv <sup>-</sup>	11	1250	370	1610	520	2860	770
conv <sup>+</sup>	6	590	190	790	210	1380	320

\* Age 30 to 35 days.

in the subepithelial tissue. Other cell types showed relatively little change. For better identification in reading the results, the affected counts are shown in boldface type in table II. Table III offers an appraisal of the significance of the differences that are discussed in this paper between various RE cell values. In these tables the following are indicated.

The gf<sup>-</sup> chickens in general displayed the lowest RE counts. In strep<sup>-</sup> birds, all cell values were essentially unchanged in comparison to gf<sup>-</sup>, with the exception of a significant increase of lymphocytes and particularly plasma cells in the subepithelial tissue.

The few clostr<sup>-</sup> chickens of the present series indicate that this microorganism is capable of increasing the RE cell count substantially. In detail, this was primarily caused by high values of Schollen leukocytes in the epithelium and high count of lymphocytes and plasma cells in the subepithelial tissue. Due to the low number of animals in these runs, *p* values were not calculated for these groups. No essential difference was found in other cell elements between clostr<sup>-</sup> and gf<sup>-</sup>.

The RE cell values of conv<sup>-</sup> birds reflected the picture described for clostr<sup>-</sup> animals in an intensified form. Thus, Schollen leukocytes, lymphocytes, and plasma cells showed a significant increase in the mentioned tissue compartments in the conv<sup>-</sup> versus gf<sup>-</sup> comparison. Among the other cell elements, a slightly higher value for macrophages was indicated in the subepithelial tissue of these animals. No clear-cut difference could be seen in other cell types between conv<sup>-</sup> and gf<sup>-</sup> birds.

Turning to the effect of antibiotic feeding, essentially unchanged values were found between gf<sup>+</sup> and gf<sup>-</sup>. In the case of all bacterially contaminated groups, penicillin as presently used resulted in lower total counts. This was caused primarily by a reduction (occasionally significant or near-significant) of those cell values that were found elevated in the untreated, contaminated groups (i.e., Schollen leukocytes in the epithelium, lymphocytes and plasma cells in the subepithelial tissue). The other differences that were observed in the RE cell count between antibiotic-treated and untreated chickens are believed to be of accidental nature.

In comparable animals of this series, a number of additional data were recorded, which in the past have been shown to reflect the absent or neutralized flora stimulation of germ-free or antibiotic-treated conventional chickens (e.g., the weight and

TABLE II

Differential Count of Scattered Reticulo-endothelial Cells Listed in Table I

		Lymphocytes			Plasma cells			Schollen leukocytes			Mono- cytes			Macro- phages			Hetero- phils			Baso- phils			Eosino- phils		
		No.	Mean	Stand. deviation of mean	Min.	Max.	Stand. deviation of mean	Min.	Max.	Stand. deviation of mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	
Epithelial tissue																									
gf <sup>-</sup>	11	218	95	356	81	—	—	116	63	189	40	1	3	0	1	12	0	0	10	42	9	31	0	1	4
gf <sup>+</sup>	6	154	72	233	57	—	—	81	38	128	34	2	8	—	0	—	—	4	14	0	6	20	0	0	—
strep <sup>-</sup>	6	178	74	385	98	—	—	183	74	385	112	3	14	0	1	3	0	0	1	2	11	20	8	1	3
strep <sup>+</sup>	6	205	101	337	94	—	—	163	101	277	66	6	9	3	0	—	—	—	—	—	12	21	2	0	—
clostr <sup>-</sup>	3	313	285	341	23	—	—	210	195	226	13	4	9	0	0	—	—	—	—	—	9	16	4	0	—
clostr <sup>+</sup>	3	196	148	254	44	—	—	57	33	78	19	1	3	0	0	—	—	1	4	0	4	7	0	0	—
conv <sup>-</sup>	11	641	247	1060	253	—	—	535	229	958	207	3	8	0	2	14	0	0	42	147	24	62	0	1	3
conv <sup>+</sup>	6	302	122	561	148	—	—	273	52	507	144	4	12	0	0	—	—	6	13	0	11	30	3	0	—
Subepithelial tissue																									
gf <sup>-</sup>	11	498	267	802	143	0	83	14	0	40	—	6	18	0	9	44	0	0	11	40	6	35	0	4	15
gf <sup>+</sup>	6	453	196	614	168	0	79	9	0	23	—	10	29	0	10	24	3	0	10	24	2	6	0	13	45
strep <sup>-</sup>	6	826	587	1060	181	269	91	22	0	51	—	26	36	19	32	50	12	0	11	23	0	—	2	5	0
strep <sup>+</sup>	6	692	458	882	161	27	166	18	0	33	—	23	41	13	18	32	5	0	8	29	0	—	11	23	0
clostr <sup>-</sup>	3	1240	945	1450	217	165	54	24	0	42	—	20	34	8	3	9	0	0	4	9	0	—	8	25	0
clostr <sup>+</sup>	3	860	604	1010	182	16	23	19	0	35	—	9	27	0	8	15	0	0	5	9	0	—	5	16	0
conv <sup>-</sup>	11	1180	585	2530	489	268	122	24	0	64	—	19	48	0	71	270	13	0	29	49	7	23	0	14	41
conv <sup>+</sup>	6	609	316	831	182	98	38	6	0	12	—	12	24	0	26	43	6	4	25	40	6	10	5	13	0

TABLE III

*Significance of Difference Between Reticulo-endothelial Cell Counts*(S:  $p < 0.01$ ; S?:  $p = 0.01-0.05$ ; O:  $p > 0.05$ )

	Schollen leukocytes in epithelial tissue				Lymphocytes in subepithelial tissue				Plasma cells in subepithelial tissue			
	gf <sup>-</sup>	gf <sup>+</sup>	strep <sup>+</sup>	conv <sup>+</sup>	gf <sup>-</sup>	gf <sup>+</sup>	strep <sup>+</sup>	conv <sup>+</sup>	gf <sup>-</sup>	gf <sup>+</sup>	strep <sup>+</sup>	conv <sup>+</sup>
gf <sup>-</sup>		0	0	S		0	S?	0		0	S?	S
strep <sup>-</sup>	0		0		S		0		S		S?	
conv <sup>-</sup>	S			S?	S			S?	S			S

cellularity of the cecal lymph nodes were found to be lower in these animals).<sup>13,14</sup> In 30 to 35 day old animals of this series (4 to 10 animals/group), the relative weight (in mg./100 Gm. body weight) and cellularity (in lymphocytes/cu. mm. native tissue) of the cecal lymph nodes were as shown in table IV. Accordingly, a certain parallelism exists between the scattered RE cell counts and the listed results.

In terms of growth (see also the paper of Wagner and Wostmann<sup>16</sup>) and general health, the animals of the present experiment showed a satisfactory picture (only gf<sup>-</sup> and gf<sup>+</sup> were somewhat underweight). On autopsy no signs of stress could be detected.

## DISCUSSION

The present results need comment on several points.

In general, the data on the reticulo-endothelial count of conv<sup>-</sup> chickens are in agreement with the qualitative observations that were described by other workers for the intestinal wall of the normal chicken.<sup>19-22</sup> The same applies to the substantially lower count of some reticulo-endothelial cells in the intestines of gf<sup>-</sup> animals of this series and the comparable data of the literature. Thus, the presently reported low lymphocyte count corresponds to the underdeveloped lymphoid system, which has been described in various germ-free species by authors mentioned in the introduction of this paper. The low values or absence of plasma cells in gf<sup>-</sup> birds generally corroborate the similar qualitative findings of Thorbecke et al.<sup>10</sup> However, there is a slight discrepancy between the two series under discussion: Thorbecke et al reported the total absence of plasma cells in the ileocecal-colic junction and ceca of ten 4 to 5 week old germ-free chickens; presently, working with the lower ileum, the same was found in only 4 of 8 comparable germ-free chickens. It is assumed that this difference is caused by the variation in experimental conditions. Among the older animals there is no difference between Thorbecke et al's results and the present results.

Another cell type that is affected by the variation of the bacterial flora is the Schollen, or globule leukocyte. This relatively little known cell, which is found mainly within epithelial tissue, has been regarded in mammals as a derivative of the lymphocyte<sup>23,24</sup> or in the chicken as a micromyelocyte.<sup>21</sup> Its role as an emigrant RE cell from the epithelium into the lumen has also been considered.<sup>24</sup> Kent et al<sup>25</sup>

TABLE IV

*Relative Weight and Cellularity of Cecal Lymph Nodes*

	gf <sup>-</sup>	gf <sup>+</sup>	strep <sup>-</sup>	strep <sup>+</sup>	clostr <sup>-</sup>	clostr <sup>+</sup>	conv <sup>-</sup>	conv <sup>+</sup>
Relative weight	10.6	13.6	20.8	14.7	25.1	13.3	51.7	24.0
Cellularity	117	189	192	155	266	135	297	149

described its marked decline or almost complete disappearance from the tracheal epithelium of the rat on administration of various amounts of corticotropin or cortisone. In another publication, Kent et al<sup>26</sup> reported considerable reduction of this cell type in the intestinal epithelium of the whole-body irradiated or hypophysectomized rat. The present observations further indicate that the Schollen leukocyte may play a role in the host versus normal contaminant relationship.

In reviewing the reticulo-endothelial elements of the intestine, the rather sizable contingent of these cells, which are consistently found in  $gf^-$  chickens, should be mentioned. From this viewpoint, the following should be considered. The diets currently fed to germ-free animals contain substances that are capable of antigenic stimulation (dead bacteria, dietary proteins<sup>27</sup>). In addition, under the presently practiced conditions of germ-free rearing, the possibility of viral or rickettsial contamination cannot be excluded. Thus the present work does not clarify the relationship of these germ-free RE cells to environmental stimulation or to phenomena of natural resistance.

*Str. faecalis* and *Cl. perfringens* have been selected as monocontaminants because former experience has indicated them to be particularly active members of the normal microbial flora.<sup>14,16</sup> This was suggested, among others, by a reduced count of these microorganisms as well as by an underdevelopment of the lymphoid system in the intestines of antibiotic-fed, conventional chickens. In the present work, the active nature of both bacterial species has been confirmed also in their role as monocontaminants. Thus an intense reticulo-endothelial stimulation (particularly by *Cl. perfringens*) became apparent almost to the level of the  $conv^-$  animal. The work of Lev et al<sup>28</sup> and Forbes et al,<sup>29</sup> dealing mainly with the growth-inhibitory effect of *Cl. perfringens* (*Clostridium welchii*) and antibiotic treatment in conventional and germ-free chickens, also points at the predominant role of this microorganism in flora stimulation.

In summation of the results to this point, it appears that germ-free life and the bacterial flora as presently studied impart a characteristic count of RE cells to the intestine of the chicken. In this qualitative effect, the same cell types are involved, irrespective of the nature of the contaminating flora.

In penicillin-treated, bacterially contaminated birds ( $strep^+$ ,  $clostr^+$ ,  $conv^+$ ), the counts of Schollen leukocytes, lymphocytes, and plasma cells in the previously mentioned tissue compartments approached more or less the levels seen in  $gf^-$  chickens. This suggests that the action of the antibiotic on the host animal was indirect, i.e., via a neutralization of the stimulatory effect of the flora (cf. Wagner and Wostmann, reduction of flora population in this series on antibiotic treatment). At the same time the lack of appreciable difference in RE cell count between the  $gf^-$  and  $gf^+$  indicates the practical absence of a direct effect of the antibiotic on the host within the limits of the present observation. This assumption naturally does not rule out the possibility of coexisting systemic effects. At this point a report of the group in Reading, England, should be mentioned;<sup>30</sup> in that study no difference was found between the intestines of comparable antibiotic-treated and untreated, conventional chickens on routine histological examination. Since the differences in RE cell count under discussion become discernible only on quantitative observation, the authors believe that these observations do not necessarily contradict the present findings. In addition, differences in the environment, diet, and bacterial flora as they exist between the animal colonies at Reading and Notre Dame may have contributed to this variance of results.

The low reticulo-endothelial cell values of the antibiotic-treated, bacterially con-

taminated chickens of this series are in unison with the reduced intestinal weight and lymphoid tissue content of the comparable animal groups previously reported:<sup>13,14</sup> in both instances they represent an approach to the characteristics of the germ-free chicken. In general, these results are consistent with the report of Coates et al.,<sup>31</sup> who first emphasized the over-all importance of the indirect (flora) effects of the antibiotic when fed to the host at nutritional levels.

Referring to the results of Wagner and Wostmann (l.c.) on serum electrophoresis and antibody titration in the present groups of monocontaminated animals, it can only be stated that the increase of reticulo-endothelial cell counts, the increase of gamma globulin, and the appearance of homologous bacterial antibodies on contamination (chiefly strep<sup>-</sup>) in comparison with the values of gf<sup>-</sup> occur as concomitant phenomena. At the same time in strep<sup>+</sup> birds, which displayed a somewhat lower plasma cell count in the intestine, gamma globulin and antibody titer of the serum were practically the same as in strep<sup>-</sup>. No explanation can be offered of this inconsistency, except that antibiotics as offered under the present circumstances may slightly reduce serum gamma globulin only on prolonged treatment, in older animals.<sup>32</sup> In addition, it must be kept in mind that at present the RE cell count was tested as a regional response, while proteins and antibodies of the serum reflect a more generalized reaction of the organism.

#### SUMMARY

1. The effects of the microbial flora and antibiotic feeding (50 mg. procaine penicillin G per Kg. diet) were studied on the scattered reticulo-endothelial cells of the intestine in germ-free, monocontaminated, and conventional chickens. These cell counts were determined in histological preparations using camera lucida and planimeter techniques.

2. A comparison between untreated germ-free and conventional chickens indicated low reticulo-endothelial cell values among the germ-free. This was particularly conspicuous in terms of Schollen leukocytes, lymphocytes, and plasma cells of the mucosa and submucosa.

3. In chickens monocontaminated with *Str. faecalis* or *Cl. perfringens*, the reticulo-endothelial cell contingent of the intestine approached the levels seen in the conventional animal.

4. On antibiotic treatment of conventional and monocontaminated birds, the number of reticulo-endothelial cells approached the germ-free values. At the same time, antibiotic treatment of germ-free chickens caused no essential change in the cell count.

5. The weight and lymphocyte concentration of the cecal lymph nodes in these animals showed changes (in terms of high and low values) that were parallel to those described for the scattered reticulo-endothelial cell count.

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#### BIBLIOGRAPHY

1. PATZELT, V.: Die Tunica Propria und die Wanderzellen der Darmschleimhaut, Handbuch der Mikroskopischen Anatomie des Menschen 5:193-214, 1936.

2. MAGNUS-ALSLEBEN, E.: Der Einfluss der Mikroorganismen auf die Vorgänge im Verdauungsstrakt beim erwachsenen Menschen, Handbuch der Normalen u. Pathologischen Physiologie 3:1027-1042, 1929.
3. GLIMSTEDT, G.: Bakterienfreie Meerschweinchen, Acta path. et microbiol. Scandinav., suppl. 30, 1936.
4. GUSTAFSSON, B.: Germ-free rearing of rats, Acta path. et microbiol. Scandinav., suppl. 73, 1948.
5. GORDON, H. A.: Morphologic characterization of germfree life, Bull. New York Acad. Med. 31:239-242, 1955.
6. GORDON, H. A.: Morphological and physiological characterization of germfree animals, Ann. New York Acad. Sc. In press.
7. MIYAKAWA, M.; IJIMA, S.; KOBAYASHI, R., AND TAJIMA, M.: Observation on the lymphoid tissue of the germfree guinea pig, Acta path. Japonica 7:183-210, 1957.
8. MIYAKAWA, M.: Le tissu lymphoïde du cobaye bactériologiquement stérile, Sang 28:698-717, 1957.
9. PHILLIPS, B. P.; WOLFE, P. A., AND GORDON, H. A.: Studies on rearing the guinea pig germfree, Ann. New York Acad. Sc. In press.
10. THORBECKE, G. J.; GORDON, H. A.; WOSTMANN, B. S.; WAGNER, M., AND REYNIERS, J. A.: Lymphoid tissue and serum gamma globulin in young germfree chickens, J. Infect. Dis. 101:237-251, 1957.
11. GORDON, H. A., AND WOSTMANN, B. S.: Responses of the animal host to changes in the bacterial environment etc., abstracts of the VII International Congress of Microbiology, Stockholm, 1958. In press.
12. POTH, E. J.: Critical analysis of intestinal antiseptics, J.A.M.A. 163:1317-1322, 1957.
13. GORDON, H. A.: Studies on the growth effect of antibiotics in germfree animals: a morphological and biochemical approach, a Colloquium, Lobund Institute, University of Notre Dame, Notre Dame, Ind., 1952.
14. GORDON, H. A.; WAGNER, M., AND WOSTMANN, B. S.: Studies on conventional and germ-free chickens treated orally with antibiotics. In: Antibiotics Annual 1957-1958, New York, Medical Encyclopedia, Inc., 1958, pp. 248-255.
15. REYNIERS, J. A.; TREXLER, P. C.; ERVIN, R. F.; WAGNER, M.; LUCKEY, T. D., AND GORDON, H. A.: Rearing Germfree Chickens, Lobund Reports no. 2, Notre Dame, Ind., University of Notre Dame, 1949, pp. 1-116.
16. WAGNER, M., AND WOSTMANN, B. S.: Studies on monocontaminated chickens (*Clostridium perfringens* or *Streptococcus faecalis*), fed penicillin. In: Antibiotics Annual 1958-1959, New York, Medical Encyclopedia, Inc., 1959, p. 1003.
17. ROMEIS, B.: Mikroskopische Technik, München, Leibnitz, 1948, p. 106.
18. Cowdry, N.: Laboratory Technique in Biology and Medicine, Baltimore, Williams & Wilkins, 1948, p. 32.
19. MUTHMAN, E.: Beiträge zur vergleichenden Anatomie des Blinddarmes und der lymphoiden Organe des Darmkanals bei Säugetieren und Vögeln, Anat. Hefte 48:65-114, 1913.
20. CLARA, M.: Beiträge zur Kenntnis des Vogeldarmes, VI, Z. f. Mikr. Anat. Forschung 6:305-350, 1926.
21. MJASSOJEDOFF, S. W.: Die Zellformen des Bindegewebes und des Blutes und die Blutbildung beim erwachsenen Huhn, Fol. haemat. 32:263-296, 1926.
22. GREULICH, R. C.: Intestinal Schollen leucocytes in the chicken, Anat. Rec. 103:571, 1949 (abstr.).
23. WEILL, P.: Über die leukozytären Elemente der Darmschleimhaut der Säugetiere, Arch. f. mikr. Anat. 93:1-81, 1920.
24. KENT, J. F.: The origin, fate and cytochemistry of the globule leucocyte of the sheep, Anat. Rec. 112:91-111, 1952.
25. KENT, J. F.; BAKER, B. L.; INGLE, D. J., AND LI, C. H.: Effect of corticotropin and cortisone on the globule leucocytes of the rat, Proc. Soc. Exper. Biol. & Med. 85:635-637, 1954.
26. KENT, J. F.; BAKER, B. L.; PLISKE, E. C., AND VAN DYKE, J. G.: Effect of X-irradiation on globule leucocytes in rat intestine, Proc. Soc. Exper. Biol. & Med. 91:152-155, 1956.
27. WAGNER, M.: Serologic observations in germfree animals, Bull. New York Acad. Med. 31:236-239, 1955.
28. LEV, M.; BRIGGS, C. A. E., AND COATES, M. E.: The gut flora of the chick. 3. Differences in caecal flora between 'infected', 'uninfected' and penicillin-fed chicks, Brit. J. Nutrition 11:354-372, 1957.
29. FORBES, M. J.; PARKS, J. Y., AND LEV, M.: Growth response and effect on intestinal flora in germfree and conventional animals fed antibiotics, Ann. New York Acad. Sc. In press.
30. COATES, M. E.; DAVIS, M. K., AND KON, S. K.: The effect of antibiotics on the intestine of the chick, Brit. J. Nutrition 9:110-119, 1955.
31. COATES, M. E.; DICKINSON, C. D.; HARRISON, G. F.; KON, S. K.; CUMMINS, S. H., AND CUTHBERTSON, W. F. J.: Mode of action of antibiotics in stimulating growth of chicks, Nature, London 168:332, 1951.
32. WOSTMANN, B. S., AND GORDON, H. A.: Electrophoretic studies on serum proteins of young germfree, conventional and antibiotic treated conventional chickens, Proc. Soc. Exper. Biol. & Med. 97:832-835, 1958.

# Antibiotic Inhibitors. III. Reversal of Calcium Inhibition of Intestinal Absorption of Oxytetracycline in Chickens by Certain Acids and Acid Salts

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Earlier investigations by the authors<sup>1,2</sup> and others<sup>3,4</sup> have established that certain multivalent cations, including calcium, exert an inhibitory effect upon the antibacterial activity of the tetracycline antibiotics. Recently, studies by Dearborn et al,<sup>5</sup> and Sweeney et al,<sup>6</sup> have shown that the simultaneous oral administration of dicalcium phosphate with tetracycline resulted in a depression in antibiotic serum levels in both rats and human beings. As a result of this finding, it is suggested by the former authors that the increase in antibiotic serum levels obtained following administration of tetracycline with citric acid may have resulted from citrate complexing of the absorption antagonist, calcium. Evidence that sodium metaphosphate, another antibiotic absorption enhancing agent, also functions through a calcium complexing mechanism has been presented by Welch and Wright.<sup>7</sup> These investigators were unable to detect any increase in oxytetracycline\* serum levels due to sodium metaphosphate when dicalcium phosphate was not employed as a capsule filler. Sodium metaphosphate in previous studies with tetracycline,<sup>8</sup> and chlortetracycline,<sup>9†</sup> where dicalcium phosphate was present in the antibiotic control capsules, produced markedly increased serum levels of these antibiotics.

In contrast to the blood serum enhancing agents just mentioned, terephthalic acid, which has been found to increase serum levels of both oxytetracycline<sup>10</sup> and chlortetracycline,<sup>11</sup> has not been shown to possess the ability to form strong complexes with calcium. The calcium-complexing hypothesis therefore would not seem to explain satisfactorily the action of this enhancing agent. The objective of the present investigation was to determine whether any of these three absorption-enhancing agents was exerting its effect through reversal of calcium inhibition of oxytetracycline absorption. In addition, other acids or acid salts with known calcium complexing ability were studied. After investigation of a number of experimental procedures, the one finally adopted was the simultaneous injection of oxytetracycline and the test compound into the ligated duodenal loop of fasted 8 to 10 week old chickens. Blood level enhancing effect was determined both in the presence and absence of exogenous calcium.

The basis for selecting this procedure was the high degree of reproducibility that could be obtained between experiments and because antibiotic serum recovery values for birds dosed via the duodenal loop had a lower coefficient of variation than did those from birds dosed by the oral route.

## EXPERIMENTAL PROCEDURE

*In Vitro Studies.* A number of inorganic and organic compounds were screened to determine their ability to reverse calcium inhibition of oxytetracycline's anti-

\* The trade name of Chas. Pfizer & Co. for oxytetracycline is Terramycin.

† The trade name of Lederle Laboratories Division, American Cyanamid Co., for chlortetracycline is Aureomycin.

bacterial activity against *Pseudomonas aeruginosa* strain 1014. Tests were conducted in Difco nutrient broth to which sufficient exogenous calcium (as hydrated calcium chloride) had been added to give a final concentration of 0.02 M. The presence of this level of cation in the medium consistently increased the minimum inhibitory concentration of oxytetracycline for the *Pseudomonas* strain from 3.2 to 25  $\mu\text{g./ml.}$  in twofold tube dilution tests. All compounds were screened by a procedure in which a geometric series of molar concentrations (ranging from 0.001 to 0.032) was employed. The degree of activity of the various test materials was determined by noting the molar concentration required to overcome completely the inhibitory effect of calcium. No attempt was made to adjust pH after addition of the test compounds, since the required hydrogen ion concentration for optimum activity varied considerably between compounds. Details of the tube dilution technique employed have been previously described.<sup>1</sup>

*Experiment 1 in Vivo.* A preliminary experiment was conducted to determine the response of chickens to oxytetracycline doses ranging from 0.16 to 10.0 mg. following their injection into the ligated duodenal loop. Blood serum, liver, and urine antibiotic levels served as direct measurements of absorption, while antibiotic recovery values from the gut wall and lumen content constituted an indirect measure of absorption. Twenty 10 week old birds (New Hampshires used in all experiments) with an average weight of 1372 Gm. were divided into 10 equal groups of comparable weight and given the graded antibiotic doses. The birds did not have access to food for approximately 18 hours prior to injection of the test solutions but did have water available until two hours pre-injection. The need to fast animals in order to obtain maximum antibiotic absorption has been shown by Dearborn et al.,<sup>5</sup> who reported that the presence of food markedly reduced blood levels of tetracycline in rats.

Ligation and injection of the duodenal loop was done as follows. The bird was anesthetized lightly with diethyl ether and then restrained on its left side with legs widely spread. Ether was then applied until the bird did not readily respond to external stimuli. A longitudinal ventral incision (2 to 3 cm.), paralleling the right thigh (approximately 4 cm. from the midline) and just posterior to the rib cage, was made and the duodenal loop exposed. Any contents were forced by gentle pressure past the posterior end of the loop. Ligatures (no. 2 silk) were applied approximately 10 cm. from the center of the loop at both the anterior (gizzard) and posterior end. Four ml. of solution containing oxytetracycline at the desired concentration was injected into the ligated loop near the anterior ligature with a 22 gauge needle. The duodenum was then replaced in the peritoneal cavity and the incision closed with Michel wound clips. Following the operation, the birds were individually housed in units with no water or food available. Pretest (30 minutes pre-injection) and one and two hour postinjection blood samples were obtained from wing veins, while three hour blood specimens were taken by heart bleeding. After the three hour samples had been collected, the birds were sacrificed and the liver and duodenum recovered. Blood samples were held at room temperature two hours or until some evidence of clot retraction was apparent and then refrigerated (4 to 6 C.) overnight. After centrifugation, the serum was decanted into test tubes and frozen at  $-40^{\circ}\text{C.}$  until assayed.

A weighed portion of the liver (approximately 5 Gm.) was placed in 2 volumes of demineralized water and ground in a Vertis homogenizer until a homogeneous suspension was obtained. This suspension was frozen at  $-40^{\circ}\text{C.}$  until assayed. The duodenal loop was incised, drained, and washed twice by allowing 2 aliquots (20

ml.) of demineralized water to pass through the lumen. The gut was then weighed and ground in 2 volumes of demineralized water and stored at  $-40^{\circ}\text{C}$ . The original loop content and the two washes were also frozen for assay. In this trial, all urine excreted during the 3 hour test was collected in beakers by use of individual cages with galvanized metal bottoms that sloped toward a central aperture. The volume of the collected urine was measured and sufficient demineralized water added, if necessary, to give 3.0 ml. The specimens were then frozen for subsequent assay.

Antibiotic concentrations in the blood serum, liver, urine, and duodenal loop (gut itself, contents, and washes) were determined in all experiments by the cylinder plate diffusion method employing *Bacillus cereus* var. *mycoides* ATCC 9634 as the test organism. Anhydrous oxytetracycline base was used as the standard of comparison. All values obtained were converted and are reported as oxytetracycline hydrochloride activity.

*Experiment 2 in Vivo.* The second experiment was concerned with selection of suitable levels of calcium and oxytetracycline for use in screening blood level enhancing agents. Twenty-four 12 week old birds of average weight 1560 Gm. (range, 1450 to 1790) were employed. Eighteen of the birds were divided into six equally weighted groups of 3. Treatments were 4 mg. oxytetracycline, 4 mg. oxytetracycline plus 16 mg. calcium, 4 mg. oxytetracycline plus 32 mg. calcium, 8 mg. oxytetracycline, 8 mg. oxytetracycline plus 16 mg. calcium, and 8 mg. oxytetracycline plus 32 mg. calcium. The remaining 6 birds were allotted into three equal groups which received 16 mg. calcium, 32 mg. calcium, or saline. All injection solutions were balanced as to ionic concentration with sodium chloride and administered in a 4.0 ml. volume.\*

Ligation and injection of the duodenal loop was carried out as described for the first experiment. Blood samples were collected 30 minutes before injection and 90 minutes postinjection from wing veins, while 180 minute specimens were taken from the heart. After the latter sample had been obtained, the birds were sacrificed and the liver and duodenal loop recovered. All specimens, including the urine excreted between 0 and 180 minutes, were handled as previously described and assayed to determine their antibiotic content. Calcium levels in the duodenal loop were measured by a method described in the Hach Chemical Company catalogue no. 3.

*Experiment 3 in Vivo.* In the next experiment, the effect of 16 mg. calcium on absorption of a 4 mg. dose of oxytetracycline was re-evaluated. Twenty-two 13 week old birds of average weight 1658 Gm. (range, 1550 to 1880) were allotted and received treatment as follows: 10 birds oxytetracycline, 4 mg.; 10 birds oxytetracycline, 4 mg. plus calcium, 16 mg.; and 2 birds, saline controls. All birds received their prescribed dose in 4.0 ml. of solution which was injected into the ligated duodenal loop. Although collection and handling of blood, liver, and duodenal loop specimens was the same as in the preceding experiment, the urine collection procedure was modified, because, in the previous experiment, urine output was erratic and specimens gave inconsistent antibiotic recovery values. It was found that urine samples could be collected directly into Petri dishes by insertion of a small speculum into the cloaca. When sample collection was made at a designated time interval after injection, excellent correlation between urine and serum anti-

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\* Although preliminary experiments indicated that injection of hypertonic solutions did not demonstrably influence absorption of oxytetracycline, in order to minimize variables, all solutions in this and subsequent experiments were balanced with sodium chloride so that a comparable osmotic effect would be obtained.

biotic levels was found. In this trial, all birds were sampled just prior to the 180 minute bleeding.

*Experiment 4 in Vivo.* The last series of experiments was concerned with the screening of various acids and salts of acids for ability to enhance oxytetracycline absorption in the presence and absence of exogenous calcium. A total of 13 experiments was conducted with 343 8 to 10 week old birds ranging in weight from 1108 to 1495 Gm. The groups of 5 or 6 birds each were treated as follows: oxytetracycline 4 mg.; oxytetracycline 4 mg. plus 0.4 or 0.8 mM of acid or salt; oxytetracycline 4 mg. plus calcium 0.4 mM plus 0.4 or 0.8 mM of acid or salt. Although selection of the acid or salt levels was somewhat arbitrary, it was not completely so, because many of the compounds being screened complex with calcium in a 1/1 or a 2/1 molecular ratio. In most of the experiments the same solutions with oxytetracycline omitted were injected into a single bird to serve as controls. In no instance did specimens from these controls show detectable antibacterial activity in assays.

Two and four hour blood and urine specimens were collected and handled as previously described. At the conclusion of each experiment (4 hours), birds were sacrificed and the liver and duodenal loop recovered. Two antibiotic assays were conducted on each serum sample, while single assay values were obtained for the liver and urine specimens. The contents of the duodenal loop were not assayed but were collected for pH determinations. Wherever applicable, the data were subjected to analysis of variance procedures. The use of the term "significant" in this paper refers to statistical significance at a probability level of 5 per cent or less.

## RESULTS

In the in vitro experiment, a number of acids or salts of acids were screened for ability to reverse calcium inhibition of oxytetracycline. The results are shown in table I. It can be noted that the tetrasodium salt of ethylenediaminetetraacetic acid (EDTA) and kojic acid were by far the most active materials under the conditions imposed by this test, since the next most active group of compounds (oxalic, orthophosphoric, and metaphosphoric acids, and sodium metaphosphate) were required at molecular concentrations four to eight times greater to bring about complete reversal of cation interference.

TABLE I

*The Relative Ability of Certain Acids and Acid Salts to Reverse Calcium Inhibition of Oxytetracycline Activity Against Pseudomonas aeruginosa (Strain 1014)*

Non-active compounds*		Molar concentration of acid or salt required to reverse completely inhibition due to 0.02 molar calcium					
		>0.032†	0.032	0.016	0.008	0.002	0.001
Malonic	Salicylic	Fumaric	PABA	Citric	Metaphosphoric	Kojic	EDTA‡
Tartaric	Na bisulfate	Triethyl citrate	Pyruvic		Sodium meta-phosphate		
Terephthalic	Benzene sulfonic	Tributyl citrate	Benzoic		Oxalic		
Phenylacetic	1,2,3, Propane tricarboxylic	Benzene phosphonic			Orthophosphoric		
Malic	Carboxymethyl cellulose	Mandelic					
Diphenylacetic							

\* No effect at 0.032 molar concentration.

† Partial reversal of calcium inhibition by 0.032 molar concentration.

‡ Tetrasodium salt of ethylenediaminetetraacetic acid.

TABLE II

*Average Blood Serum, Urine, and Liver Levels Produced by Injection of Various Doses of Oxytetracycline Hydrochloride into the Ligated Duodenal Loop of Chickens*

Oxytetracycline injected, mg.	Serum levels, $\mu\text{g.}/\text{ml.}$				Urine levels, $\mu\text{g.}/\text{ml.}$ , 0-180	Liver levels, $\mu\text{g.}/\text{Gm.}$ , 180
	60*	120	180	Av.		
0.16	<0.162	<0.162	—	<0.162	—	—
0.30	<0.162	<0.209	0.207	0.193	9.0	0.570
0.90	0.218	0.225	0.297	0.247	9.93	0.797
1.50	0.221	0.272	0.264	0.252	7.97	0.803
2.00	0.378	0.205	0.273	0.285	15.26	0.924
4.00	0.473	0.409	0.421	0.434	40.37	1.110
6.00	0.630	0.640	0.496	0.589	15.71	1.464
8.00	0.719	0.740	0.753	0.737	91.26	1.968
10.00	0.803	0.706	0.912	0.807	61.48	2.357

\* Time in minutes.

Citric, pyruvic, benzoic, and *p*-aminobenzoic acids also completely reversed calcium inhibition, although at somewhat higher levels. All remaining compounds had little or no effect at the 0.032 molar level. It is of interest that terephthalic acid was among those which failed to show ability to influence calcium antagonism toward oxytetracycline in this experiment.

In the first in vivo experiment, the dosage response of chickens to graded levels of oxytetracycline was determined. Results are shown in table II. Although the minimum dose giving measurable levels at all three time periods was 0.90 mg., detectable levels were present at 120 and 180 minutes with the 0.30 mg. dose. It is apparent that, in the dosage range studied, a fairly consistent rise in blood serum concentration was obtained with increasing dosage level (see figure 1). A similar response was noted with liver specimens, where antibiotic levels were in a range two to three times higher than those of the serum samples. Although results from urine specimens were somewhat inconsistent, it can be noted that antibiotic concentrations tended to increase as the dosage level was raised.

The total antibiotic recovered from the lumen fluid, gut washes, and gut wall is shown in table III. The lumen fluid and gut wash recovery values were combined to give the figures shown in the column headed "Lumen Content Recovery." It is apparent that almost all of the unabsorbed antibiotic was present in the lumen fluid, since recoveries from the gut wall were low. However, it would appear that

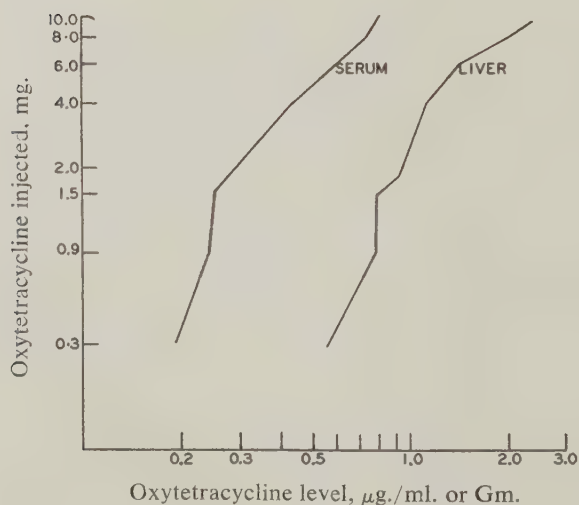


FIG. 1. Serum and liver oxytetracycline levels following injection into ligated duodenal loop of chicks.

TABLE III

*Average Antibiotic Recovery from the Duodenal Loop 180 Minutes Following Injection of Various Oxytetracycline Doses*

Quantity injected, mg.	Lumen content recovery,* mg.	Gut wall recovery, mg.	Total recovered, mg.	Per cent absorbed†
0.16	0.121	0.007	0.128	19.8
0.30	0.201	0.009	0.210	30.0
0.90	0.460	0.022	0.482	45.5
1.50	0.922	0.048	0.970	34.3
2.00	1.490	0.033	1.523	23.9
4.00	2.095	0.123	2.218	44.5
6.00	4.419	0.113	4.532	24.5
8.00	5.942	0.144	6.086	24.0
10.00	6.892	0.363	7.255	27.4

\* Includes antibiotic in washes.

† Total oxytetracycline injected minus total recovered  $\times 100$

Total injected

the concentration in the wall itself was dependent on the level of antibiotic injected. Examination of the total recovery values shows that, in all cases, the amount of oxytetracycline recovered increased as the dosage level was raised. The percentage of absorbed antibiotic (injected quantity minus the recovered quantity) ranged from 19.8 to 45.5, and averaged 30.5.

When the quantity of antibiotic injected is plotted against quantity recovered (fig. 2) a nearly linear relationship for doses between 0.16 and 10.0 mg. is shown.

The influence of two calcium levels (16 and 32 mg.) on absorption of 4 and 8 mg. doses of oxytetracycline was determined in experiment 2. These levels were selected because it had been shown in *in vitro* studies,<sup>1</sup> that at least 50 mM of cation are required to reverse the antibacterial activity of 1 mM of oxytetracycline. Sixteen mg. calcium and 4 mg. oxytetracycline constitute a molecular ratio of approximately 50/1. The average blood, urine, and liver antibiotic concentrations found in this trial are presented in table IV.

These data show that calcium substantially reduces levels of oxytetracycline in blood serum, urine, and liver when injected simultaneously with the antibiotic into the duodenal loop. It appears that, with 4 mg. of antibiotic, maximum inhibition was

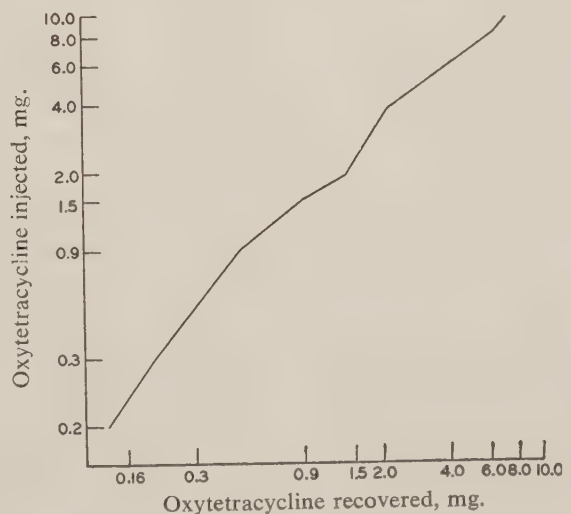


FIG. 2. Oxytetracycline recovered from ligated duodenal loop of chicks four hours after injection.

TABLE IV

*Blood Serum, Urine, and Liver Oxytetracycline Levels Following Injection of the Antibiotic into the Ligated Duodenal Loop in the Presence and Absence of Exogenous Calcium\**

Treatment	Oxytetracycline level		
	Serum, $\mu$ g.	Urine, $\mu$ g.	Liver, $\mu$ g.
Oxytetracycline, 4 mg.	0.558	18.4	1.185
Oxytetracycline, 4 mg. plus calcium, 16 mg.	0.209	5.7	0.742
Oxytetracycline, 4 mg. plus calcium, 32 mg.	0.263	8.6	0.905
Oxytetracycline, 8 mg.	0.734	96.1	1.290
Oxytetracycline, 8 mg. plus calcium, 16 mg.	0.489	21.6	0.920
Oxytetracycline, 8 mg. plus calcium, 32 mg.	0.310	35.7	0.716
Calcium, 16 mg.	<0.162	<0.2	<0.486
Calcium, 32 mg.	<0.162	<0.2	<0.486
Sodium chloride control	<0.162	<0.2	<0.486

\* Average of 90 and 180 minute postinjection samples.

obtained with 16 mg. of calcium, since a twofold increase in cation concentration did not cause further reduction in antibiotic serum levels. However, with the 8 mg. oxytetracycline dose, there was some evidence that 16 mg. calcium did not give maximum suppression, as both serum and liver levels were higher than those obtained with 32 mg. calcium. Urine values were not consistent with this trend. However, as previously pointed out, the sample collection method used in this and the preceding experiment gave results that were much less reliable than those obtained with serum and liver specimens. No detectable antibiotic level was present in specimens from control birds which had received only calcium or sodium chloride.

The quantity of antibiotic and calcium recovered from the duodenal loop of birds on the various treatments is shown in table V. It would appear that the endogenous calcium content of the duodenum is approximately 5 mg. under these conditions. This value, of course, includes the calcium present in both the lumen fluid and gut wall. If one assumes that the same amount of endogenous calcium is present in the duodenum of all birds, 5 mg. should be subtracted from the total amount recovered in order to estimate more accurately the quantity of unabsorbed exogenous calcium. When this adjustment is made, it would appear that approximately 50 per cent of the exogenous calcium was absorbed at both the 16 and 32 mg. dosage level. Presence of antibiotic did not have any obvious effect on absorption

TABLE V

*Average Oxytetracycline and Calcium Recovery from the Ligated Duodenal Loop 180 Minutes after Injection of Various Quantities*

Treatment	Calcium recovery		Oxytetracycline recovery	
	mg.	Per cent (adjusted)*	mg.	Per cent
Oxytetracycline, 4 mg.	5.13	—	3,487	87.2
Oxytetracycline, 4 mg. plus calcium, 16 mg.	12.79	48.7	3,600	90.0
Oxytetracycline, 4 mg. plus calcium, 32 mg.	21.14	50.4	3,650	91.3
Oxytetracycline, 8 mg.	4.88	—	8,822	110.3
Oxytetracycline, 8 mg. plus calcium, 16 mg.	10.93	43.3	8,170	102.1
Oxytetracycline, 8 mg. plus calcium, 32 mg.	24.26	60.2	8,760	109.5
Calcium, 16 mg.	12.27	45.4	—	—
Calcium, 32 mg.	27.24	69.5	—	—
Sodium chloride control	5.49	—	—	—

\* Five mg. deducted for endogenous calcium.

of calcium. Bird-to-bird variation was quite high for recovery of antibiotic from the duodenum, especially at the higher dose (8 mg.). No difference in recovery due to the presence of calcium was indicated.

In experiment 3 confirmation was obtained that 16 mg. of calcium markedly inhibits absorption of 4 mg. oxytetracycline as suggested by the data in experiment 2. Sufficient birds were employed per group to allow statistical treatment of data. The results are summarized in table VI. A significant depression in the 90 and 180 minute serum oxytetracycline levels occurred due to the presence of 16 mg. calcium. Confirmation of suppressed absorption was provided by the urine assay values, where a corresponding depression in oxytetracycline concentration was found. The excellent correlation existing between serum and urine oxytetracycline levels indicated that collection of urine specimens coincident with serum sampling was much superior to collection of total urine over the whole test period. As would be anticipated, the lower oxytetracycline blood levels obtained from birds receiving calcium treatment resulted in significantly lower liver levels. It can also be noted that a greater recovery of antibiotic was made from the loops of birds receiving calcium, although the difference could not be demonstrated to be significant. Greater recovery, of course, would be expected if less antibiotic is being absorbed from the duodenum of calcium treated birds.

In experiment 4, three organic acids, three salts of organic acids, and one salt of an inorganic acid were screened for ability to enhance oxytetracycline absorption in the presence and absence of exogenous calcium. Table VII shows the effect of citric, kojic, and terephthalic acids on blood serum, urine, and liver levels of oxytetracycline. It can be noted that the antibiotic levels of specimens from the control group which received oxytetracycline only were fairly consistent in this series of trials. Average serum concentrations, for example, had a range of only 0.54 to 0.70  $\mu\text{g.}/\text{ml.}$  Citric acid at the 0.4 and 0.8 mM level in the absence of exogenous calcium fail to alter significantly specimen antibiotic levels. Here, with the exception of liver concentrations at the lower acid level, all responses were of extremely small magnitude. Calcium caused a marked depression in serum, liver, and urine antibiotic concentrations. Only the reduction in liver levels was not significant.

The last column shows the activity of citric acid in the presence of 0.4 mM of exogenous calcium. It can be noted that the lower citric acid level had the ability to overcome partially the depression in oxytetracycline absorption caused by calcium. For example, in the case of serum, the depression was decreased from 0.27 to 0.14  $\mu\text{g.}/\text{ml.}$  Liver and urine levels were restored to approximately the levels which occurred in the absence of calcium. Although both serum and liver level increases

TABLE VI

*The Influence of Exogenous Calcium on Absorption of Oxytetracycline from the Ligated Duodenal Loop of Chickens*

Specimen	Oxytetracycline/ml. or Gm.	
	4 mg. oxytetracycline	4 mg. oxytetracycline plus 16 mg. calcium
Serum (90 min. sample)	0.414	0.162*
Serum (180 min. sample)	0.351	0.212*
Liver (180 min. sample)	0.807	0.563*
Urine (180 min. sample)	60.0	20.6*
Duodenal loop	3301.9†	3686.6†

\* Significant decrease from oxytetracycline.

† Unabsorbed oxytetracycline.

TABLE VII

*Influence of 3 Organic Acids on Oxytetracycline Absorption from the Duodenal Loop in the Presence and Absence of Exogenous Calcium*

Acid	Acid concentration, mM	Specimen	Sampling time, hr.	Specimen oxytetracycline content, $\mu\text{g.}/\text{ml.}$ or Gm.			
				Oxytetra-cycline, 4 mg.	Oxytetra-cycline, 4 mg. plus acid	Oxytetra-cycline, 4 mg. plus calcium 0.4 mM (16 mg.)	Oxytetra-cycline, 4 mg. plus calcium 0.4 mM plus acid
Citric	0.4 (76.8 mg. citric, 66.4 mg. terephthalic)	Serum	2 & 4	0.67	0.71	0.40°	0.53°
		Liver	4	1.06	1.39	0.88	1.04
		Urine	2 & 4	68.10	75.80	36.30°	62.40†
	0.8 (153.6 mg. citric, 113.6 mg. kojic, 132.8 mg. terephthalic)	Serum	2 & 4	0.54	0.56	0.37°	0.55†
		Liver	4	1.47	1.35	1.14	1.45
		Urine	2 & 4	81.00	68.00	26.80°	71.00†
Kojic	0.8	Serum	2 & 4	0.54	0.58	0.32°	0.27°
		Liver	4	1.32	1.30	0.75°	0.81°
		Urine	2 & 4	75.60	65.30	25.20°	21.90°
Terephthalic	0.4	Serum	2 & 4	0.54	0.81†	0.28°	0.36
		Liver	4	1.12	1.64†	0.85°	1.02
		Urine	2 & 4	87.20	50.00§	26.70°	21.50°
	0.8	Serum	2 & 4	0.70	1.03†	0.27°	0.53°†
		Liver	4	1.10	1.42†	0.80°	0.93
		Urine	2 & 4	81.80	58.90	42.40°	31.50°

° Significant decrease from oxytetracycline.

† Significant increase over oxytetracycline.

‡ Significant increase over oxytetracycline plus calcium.

§ Significant decrease from oxytetracycline due to acid.

approached significance, only urine specimens had significantly higher concentrations than those found in the presence of calcium and no citric. At the higher citric acid level, complete reversal of calcium inhibition was obtained, all specimen levels approximating those of the group which received oxytetracycline only.

Kojic acid was evaluated only at the 0.8 mM level. It failed to influence oxytetracycline concentrations either in the absence or presence of exogenous calcium.

Terephthalic acid, in contrast to citric acid, significantly enhanced oxytetracycline serum and liver concentrations in the absence of exogenous calcium, increases approaching 50 per cent being obtained at both levels of acid. In the presence of calcium, some ability to enhance oxytetracycline serum and liver levels was noted at the lower terephthalic acid concentration, while a significant enhancement of calcium-depressed serum levels was obtained with the higher concentration of acid. It can be observed that, at both levels of terephthalic acid, a depression in urine antibiotic level was obtained. This was significant at the lower terephthalic level in the absence of exogenous calcium. Statistical analysis of the two levels combined showed that a significant depression in urinary levels occurred both in the presence and absence of added calcium.

Table VIII shows the results obtained when sodium metaphosphate, the tetrasodium salt of EDTA, potassium oxalate, and methenamine mandelate were tested for ability to enhance oxytetracycline specimen levels.

Sodium metaphosphate slightly increased oxytetracycline serum and urine levels in the absence of added calcium. Liver levels were slightly higher than in the group

receiving only oxytetracycline in one instance, lower in the other. The expected marked inhibition of oxytetracycline absorption by calcium was obtained in both trials, significantly lower antibiotic concentrations being recorded in serum, liver, and urine specimens. When tested in the presence of calcium, sodium metaphosphate appeared to cause some reversal of the decreased absorption due to the cation, although a significant effect was not obtained at either level of the salt when separate analyses were made. An analysis of the combined data from the two trials showed that this salt significantly enhanced serum levels in the presence of calcium as compared to those of the group receiving oxytetracycline and calcium. No significant effect on urine or liver levels could be shown.

EDTA was tested only at the 0.4 mM level. Although it was without effect in the absence of added calcium, it caused a significant enhancement of oxytetracycline absorption in the presence of calcium. This was reflected by increases in the blood serum, liver, and urine antibiotic concentrations over those of the group receiving oxytetracycline plus calcium.

Potassium oxalate proved itself to be perhaps the most effective of the calcium complexing agents tested, since it completely overcame the effect of 0.4 mM of calcium at an equimolar concentration. As with EDTA, potassium oxalate failed to give any increase in specimen levels when exogenous calcium was not present.

The last salt tested, methenamine mandelate, did not influence serum, urine, or liver oxytetracycline concentrations at the 0.4 mM level, either in the presence or

TABLE VIII  
*The Influence of 4 Acid Salts on Oxytetracycline Absorption from the Duodenal Loop in the Presence and Absence of Exogenous Calcium*

Acid	Salt concentration, mM	Specimen	Sampling time hr.	Specimen oxytetracycline content, µg./ml. or Gm.			
				Oxytetra-cycline, 4 mg.	Oxytetra-cycline, 4 mg. plus salt	Oxytetra-cycline, 4 mg. plus calcium 0.4 mM (16 mg.)	Oxytetra-cycline, 4 mg. plus calcium 0.4 mM plus salt
Sodium metaphosphate	0.4*	Serum	2 & 4	0.52	0.61	0.23†	0.29
		Liver	4	1.14	1.00	0.62†	0.65
		Urine	2 & 4	54.80	64.25	17.00†	20.50†
	0.8†	Serum	2 & 4	0.57	0.65	0.24†	0.36
		Liver	4	1.10	1.16	0.77†	0.86†
		Urine	2 & 4	75.20	76.20	33.00†	52.00†
Tetrasodium EDTA	0.4	Serum	2 & 4	0.61	0.62	0.31†	0.42§
		Liver	4	1.16	1.17	0.73†	1.01§
		Urine	2 & 4	70.70	73.1	40.90†	54.40§
Potassium oxalate	0.4	Serum	2 & 4	0.54	0.51	0.35†	0.54§
		Liver	4	1.30	1.30	0.98†	1.26§
		Urine	2 & 4	44.80	40.10	30.80	52.40§
Methenamine mandelate	0.4	Serum	2 & 4	0.50	0.55	0.31†	0.35†
		Liver	4	1.50	1.30	0.96†	1.01
		Urine	2 & 4	77.40	61.50	47.30†	54.40
	0.8	Serum	2 & 4	0.54	0.59	0.35†	0.49†
		Liver	4	1.19	1.12	0.87†	1.04
		Urine	2 & 4	44.80	48.10	30.80	30.80

\* 40.8 mg. sodium metaphosphate, 152.1 mg. tetrasodium EDTA, 73.6 mg. potassium oxalate, and 116.8 mg. methenamine mandelate.

† 81.6 mg. sodium metaphosphate and 233.6 mg. methenamine mandelate.

‡ Significant decrease from oxytetracycline.

§ Significant increase over oxytetracycline plus calcium.

absence of added calcium. However, a significant effect on calcium inhibition of antibiotic absorption was obtained at the higher methenamine level, as indicated by the increase in blood serum concentration as compared with that in the group receiving oxytetracycline plus calcium. A similar, but nonsignificant effect on liver oxytetracycline level was evident. The urine antibiotic concentration failed to increase correspondingly with the serum level in this trial.

The ability of the duodenum to buffer acid solutions was regularly apparent in this series of trials. With two exceptions, all duodenal loop contents at four hours postinjection had average *pH* values that ranged from 6.7 to 7.1 and did not differ significantly from those of the saline controls. This occurred despite the fact that some of the injected solutions had *pH* values of 3.0 or less. The two exceptions were loop contents following injection of 0.8 mM of citric and terephthalic acids. The average final *pH* values for these were approximately 0.5 units lower than those that had received injection of oxytetracycline only.

#### DISCUSSION

Absorption of injected oxytetracycline from the ligated duodenal loop of chickens was in linear relation to dose in the range from 0.16 to 10.0 mg. A similar relationship was found by Gray et al,<sup>12</sup> when oral doses ranging from 5 to 25 mg./Kg. were administered to intact dogs.

In the present study, antibiotic absorbed at the various dosage levels ranged from 19.8 to 45.5 per cent and averaged 30.5 per cent. The latter percentage approximated that obtained in human beings by Sweeney et al,<sup>6</sup> who administered a single 250 mg. capsule of tetracycline hydrochloride and determined total urinary excretion as a measurement of absorption. The average quantity of antibiotic absorbed was found to be in the range of 30 to 40 per cent of the administered dose.

Administration of calcium simultaneously with oxytetracycline in the present study markedly suppressed antibiotic absorption as manifested by decreased blood serum, liver, and urine levels. It is of interest that the minimum molecular ratio of cation to antibiotic (50/1) that inhibited the antibacterial activity of oxytetracycline *in vitro*<sup>1</sup> also was capable of suppressing antibiotic absorption *in vivo*. A suggestion that maximum inhibition of absorption was being obtained at this ratio was given by the fact that a 100/1 ratio of cation to antibiotic failed to cause any further reduction.

Relatively constant serum, liver, and urine oxytetracycline levels were obtained both within a given experiment and between experiments following injection of a 4 mg. dose. This, and the equally consistent depression in specimen levels following administration of 16 mg. of calcium, permitted one readily to determine whether antibiotic serum level enhancing agents were exerting their effect through reversal of cation inhibition. The coefficient of variation for antibiotic levels in serum samples from birds receiving a given treatment ranged from 20 to 48 per cent and averaged 31 per cent for all experiments. In trials in which birds were individually dosed by the oral route, coefficients of variation as high as 100 per cent were common.<sup>10</sup>

Citric acid and sodium metaphosphate, which are now being extensively used as adjuvants to enhance tetracycline serum levels, were both found to increase oxytetracycline absorption only where it had been suppressed by calcium. Since both compounds are known to form strong complexes with calcium and were shown to be capable of reversing cation inhibition of oxytetracycline in *in vitro* studies, this finding was not too surprising. Similarly, potassium oxalate and the tetrasodium salt of

EDTA showed marked ability to reverse calcium inhibition of oxytetracycline both in vitro and in vivo. Although one might anticipate that any compound having the ability to reverse calcium inhibition of oxytetracycline in vitro would be capable of increasing absorption of antibiotic in the presence of this cation, kojic acid failed to do so. However, since it is known that calcium kojic chelates are not so strong as those of calcium and EDTA, it may be that under the conditions present in the ligated duodenal loop, kojic acid is unable to maintain the chelate.

Methenamine mandelate was included in this study because it had demonstrated moderate ability to enhance oxytetracycline serum levels in chickens when administered by the oral route.<sup>10</sup> The enhancing effect observed in the present study may have been due to the mandelic acid portion of the molecule since this acid was shown to have some influence, although moderate, on calcium inhibition of oxytetracycline in vitro.

The influence of endogenous calcium on oxytetracycline absorption would appear to be minor, as evidenced by the small responses obtained with calcium complexing and sequestering agents in the absence of added calcium. However, the fact that most of the compounds gave a slight (although not significant) increase in serum levels in the absence of exogenous calcium suggests that endogenous calcium was not completely without effect.

The mode of action of terephthalic acid is obviously unrelated to that of the discussed compounds. Its action was completely independent of calcium, inasmuch as it enhanced serum and liver levels both in the presence and absence of exogenous calcium.

The significant depression of urine antibiotic content following administration of terephthalic acid offers a possible clue to the mode of action of the compound. It may be that the enhancement of serum and liver levels occurred because terephthalic acid alters renal clearance of the antibiotic. This possibility will be considered further in a later publication.

#### SUMMARY AND CONCLUSIONS

1. Doses of oxytetracycline ranging from 0.16 to 10.0 mg. were absorbed in a nearly linear fashion from the ligated duodenal loop of chickens.

2. Absorption of oxytetracycline from the duodenal loop was markedly inhibited by calcium, as evidenced by depressed serum, urine, and liver antibiotic levels.

3. Citric acid, sodium metaphosphate, potassium oxalate, methenamine mandelate, and the tetrasodium salt of ethylenediaminetetraacetic acid were capable of bringing about partial to complete reversal of the depression in oxytetracycline absorption caused by exogenous calcium.

4. Kojic acid did not influence absorption of oxytetracycline in the presence or absence of exogenous calcium.

5. Terephthalic acid increased oxytetracycline serum and liver levels in the presence or absence of exogenous calcium. Urine antibiotic levels were found to be depressed.

#### BIBLIOGRAPHY

1. PRICE, K. E.; ZOLLI, Z., JR.; ATKINSON, J. C., AND LUTHER, H. G.: Antibiotic inhibitors. I. The effect of certain milk constituents, *Antib. & Chemo.* 7:672-688, 1957.
2. PRICE, K. E.; ZOLLI, Z., JR.; ATKINSON, J. C., AND LUTHER, H. G.: Antibiotic inhibitors. II. Studies on the inhibitory action of selected divalent cations for oxytetracycline, *Antib. & Chemo.* 7:689-701, 1957.

3. WEINBERG, E. D.: The reversal of the toxicity of oxytetracycline (Terramycin) by multivalent cations, *J. Infect. Dis.* 95:291, 1954.
4. SONCIN, E.: Fenomeni di interferenza tra elettrolita e antibiotici. III. Ione magnesio e aureomicina, Terramicina, chloramfenicolo, *Arch. Int. Pharmacodyn.* 94:346, 1953.
5. DEARBORN, E. H.; LITCHFIELD, J. T., JR.; EISNER, H. J.; CORBETTE, J. J., AND DUNNETT, C. W.: The effects of various substances on the absorption of tetracycline in rats, *AM & CT* 4:627-641, 1957.
6. SWEENEY, W. M.; HARDY, S. M.; DORNBUSH, A. C., AND RUEGSEGG, J. M.: Absorption of tetracycline in human beings as affected by certain excipients, *AM & CT* 4:642-656, 1957.
7. WELCH, H., AND WRIGHT, W. W.: Effects of citric acid and sodium hexametaphosphate on serum concentrations from orally administered oxytetracycline hydrochloride, *AM & CT* 4:735-739, 1957.
8. WELCH, H.; WRIGHT, W. W., AND KIRSHBAUM, A.: A comparison of blood concentrations following oral administration of tetracycline hydrochloride, tetracycline base, and tetracycline base mixed with metaphosphate, *AM & CT* 4:293-296, 1957.
9. WELCH, H.; WRIGHT, W. W., AND LEWIS, C. N.: Blood concentrations following oral administration of chlortetracycline hydrochloride and chlortetracycline base, with and without sodium hexametaphosphate, *AM & CT* 4:414-418, 1957.
10. ANON.: Chas. Pfizer & Co., Inc., unpublished data.
11. PETERSON, E. H.: Potentiating effect of terephthalic acid upon absorption of chlortetracycline from the avian alimentary tract, Report Series 74 Agric. Exper. Sta., University of Arkansas, 1958.
12. GRAY, W. D.; HILL, R. T.; WINNE, R., AND CUNNINGHAM, R. W.: Enhancement of chlortetracycline absorption by citric acid, *J. Pharmacol. & Exper. Therap.* 110:327-333, 1954.

# The Effect of Gamma Globulin on Antibiotic Requirements for Control of Standardized Infections in Mice

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Recent investigations have shown that gamma globulin can supplement antibiotics used in the control of experimental infections.<sup>1-3</sup> There is evidence that this action also operates in clinical therapy.<sup>4,5</sup>

The present report is concerned with quantitative evaluations of the effectiveness of gamma globulin in antibiotic control of four experimental infections in mice. Several commercial preparations of this blood fraction were compared with respect to their action in tetracycline and chloramphenicol therapy.

## EXPERIMENTAL

The animals used in these studies were male albino mice, strain CF1, supplied by Carworth Farms. They weighed 18 to 22 Gm. each and were used in unit test groups of 10 mice/cage.

Lethal infections were produced by intra-abdominal injection of the following bacteria, using appropriate dilutions of the blood broth cultures: *Diplococcus pneumoniae*, strain SV1,  $10^{-6}$ ; *Staphylococcus aureus*, strain Smith,  $10^{-2}$ ; *Streptococcus pyogenes*, strain C203,  $10^{-5}$ . The infecting dose was contained in 0.5 ml. The mortality rates for the untreated, infected controls were greater than 95 per cent, with an average survival time of one or two days, depending on the infecting organism.

A nonlethal infection was produced by the subcutaneous injection of *Staph. aureus*, strain Rose, using a  $10^{-2}$  broth dilution of a five hour blood broth culture. The infecting dose was 0.5 ml./mouse. This inoculation produced skin abscesses in 98 per cent of the untreated infected mice within 10 days.

Commercial preparations (manufacturers A, B, and C\*) of human immune globulin ( $16 \pm 1.5$  per cent gamma globulin) were diluted in saline and administered subcutaneously or intra-abdominally in 0.5 ml. portions one half hour after infection, unless otherwise stated. The dilutions of gamma globulin used (1 to 4 or greater) were such as to provide no significant protection when administered to the infected mice without antibiotic.

Chloramphenicol was suspended and tetracycline was dissolved in 0.2 per cent aqueous agar in amounts that would produce the desired dose in 0.5 ml. of the preparation. For each test, several dosages of antibiotics graded on a two- or four-fold scale were used. They were administered by the subcutaneous, oral, or intra-abdominal route approximately one hour after infection. The mice were observed for survival for 14 days after infection.

Results of replicate tests, involving 20 mice or more at each dosage level, were pooled and the median effective dose ( $ED_{50}$ ) of the antibiotic was determined by the procedure of Litchfield and Wilcoxon for evaluating dosage-response experiments. The  $ED_{50}$  of the antibiotic when administered alone was compared with the  $ED_{50}$  of the antibiotic when administered to mice treated with gamma globulin.

\* Manufacturer A = Lederle Laboratories Division, American Cyanamid Co.; B = Parke, Davis & Co.; C = Cutter Laboratories.

TABLE I  
Streptococcus C203 Infection in Mice:  
Effect of Gamma Globulin on the ED<sub>50</sub> of Tetracycline or Chloramphenicol

Gamma globulin*	Antibiotic		
	ED <sub>50</sub> , <sup>†</sup> mg./Kg.	Slope <sup>‡</sup> function	Relative potency
<i>Tetracycline (subcutaneously)</i>			
None	28 (15-53)	5.8 (2.5-13)	1.0
A-3	15 (8-28)	6.6 (3.3-13)	1.9 (0.8-4.7)
B-1	18 (9-36)	7.6 (3.2-18)	1.6 (0.6-4.0)
<i>Chloramphenicol (subcutaneously)</i>			
None	320-640		
A-3	160-640		
B-1	160-640		

\* Gamma globulin diluted 1 to 4 in saline, 0.5 ml./mouse injected subcutaneously ½ hour after infection. This treatment alone was without significant effect.

<sup>†</sup> Figures in parentheses are 95 per cent confidence limits.

<sup>‡</sup> Slope function =  $\frac{ED_{50}/ED_{10} + ED_{50}/ED_{90}}{2}$ .

## RESULTS

*Str. pyogenes, Strain C203, Infection.* Two different commercial preparations of gamma globulin did not modify to a statistically significant degree the median effective dosage of tetracycline for this infection (table I). The data for chloramphenicol, with or without gamma globulin, did not permit the calculation of the two ED<sub>50</sub> values because of lack of response data above the 70 per cent level. The results on which the ED<sub>50</sub> of tetracycline and the ED<sub>50</sub> ranges of chloramphenicol are based are shown in table II. They show that the use of gamma globulin in the chloramphenicol treatment of mice infected with streptococci produced no significant beneficial effect.

*Staph. aureus, Strain Smith, Infection.* The median effective dosages of tetra-

TABLE II  
Effect of Tetracycline and Chloramphenicol Administered with and without Gamma Globulin on the Control of a Streptococcus C203 Infection in Mice  
(Gamma Globulin Subcutaneously, Antibiotic Subcutaneously)

Antibiotic, single dose subcutaneously, mg./Kg.	Tetracycline						Chloramphenicol					
	with gamma globulin (1 to 4)						with gamma globulin (1 to 4)					
	Alone	A-3		B-1			Alone	A-3		B-1		
	S.R.*	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect	S.R.	% effect
640							11/20	55	12/20	60	14/20	70
320	19/20	95	9/10	90	10/10	100	6/20	30	11/20	55	10/20	50
160							3/20	15	7/20	35	8/20	40
80	13/20	65	18/20	90	17/20	85			4/10	40	5/10	50
40												
20	9/20	45	9/20	45	8/20	40						
10												
5	3/20	15	8/20	40	6/20	30						
2.5												
1.25			0/10	0	1/10	10						

Survival ratio for controls: Gamma globulin alone—(A-3) 1/20; (B-1) 0/20. Infected, untreated controls—1/40.

\* Survival ratio = number of mice alive on the fourteenth day after infection/total number tested.

TABLE III

*Staphylococcus, Smith, Infection in Mice:*  
*Effect of Gamma Globulin on the ED<sub>50</sub> of Tetracycline and Chloramphenicol*

Gamma globulin*	Antibiotic		
	ED <sub>50</sub> , mg./Kg.	Slope function	Relative potency
<i>Tetracycline (orally)</i>			
None	8 (6-10)	3.1 (2.3-4.2)	1.0
A-3	4 (4-6)	2.9 (2.0-4.2)	2.0 (1.2-2.7)
<i>Tetracycline (subcutaneously)</i>			
None	1.5 (1.2-2.0)	1.8 (1.5-2.1)	1.0
A-3	* 0.7 (0.5-1.1)	2.5 (1.7-3.6)	2.1 (1.3-3.3)
B-1	1.3 (1.0-1.8)	2.3 (1.7-3.1)	1.2 (0.8-1.8)
<i>Chloramphenicol (orally)</i>			
None	88 (66-120)	2.7 (2.0-3.7)	1.0
A-3	61 (45-82)	2.3 (1.7-3.0)	1.4 (0.9-2.1)
<i>Chloramphenicol (subcutaneously)</i>			
None	110 (73-150)	2.8 (1.9-4.2)	1.0
A-3	52 (39-70)	2.0 (1.5-2.7)	2.1 (1.3-3.4)
B-1	72 (53-97)	2.0 (1.5-2.7)	1.5 (0.9-2.4)

\* Gamma globulin diluted 1 to 4 with saline; 0.5 ml./mouse injected subcutaneously ½ hour after infection. This treatment alone was without significant effect.

cycline and chloramphenicol administered alone or along with gamma globulin to mice infected with this organism are shown in table III.

When gamma globulin, preparation A-3, was used in conjunction with tetracycline, a twofold decrease in both the subcutaneous and oral ED<sub>50</sub> of the latter resulted. In conjunction with chloramphenicol, a twofold decrease in subcutaneous ED<sub>50</sub> but no decrease in oral ED<sub>50</sub> was observed.

A sample of gamma globulin, preparation B-1, however, when used in conjunction with either tetracycline or chloramphenicol, produced no change in the subcutaneous ED<sub>50</sub> of either antibiotic.

*Staph. aureus, Strain Rose, Infection.* In vitro tests have shown that *Staph. aureus*, strain Rose, is resistant to tetracycline, but sensitive to chloramphenicol. This sensitivity pattern was also observed in vivo since even high dosages of tetracycline did not prevent in mice the appearance of skin abscesses resulting from this

TABLE IV

*Staph. aureus, Strain Rose, Infection in Mice:*  
*Effect of Gamma Globulin on the ED<sub>50</sub> of Tetracycline and Chloramphenicol*  
*(Gamma Globulin Intra-abdominally, Antibiotic Orally)*

Gamma globulin*	Antibiotic		
	ED <sub>50</sub> , mg./Kg.	Slope function	Relative potency
<i>Tetracycline</i>			
None	>640		
A-3	>640		
<i>Chloramphenicol</i>			
None	70 (47-100)	3.1 (1.8-5.4)	1.0
A-3	52 (36-76)	2.3 (1.5-3.5)	1.3 (0.8-2.2)

\* Gamma globulin diluted with saline 1 to 4; 0.5 ml./mouse intra-abdominally ½ hour after infection. This treatment alone was without significant effect.

TABLE V  
D. pneumoniae, Strain SVI, Infection in Mice:  
Effect of Gamma Globulin on the ED<sub>50</sub> of Tetracycline and Chloramphenicol

Gamma globulin*	Antibiotic		
	ED <sub>50</sub> , mg./Kg.	Slope function	Relative potency
<i>Tetracycline (orally)</i>			
None	280 (190-410)	2.9 (1.7-4.9)	1.0
A-3	78 (58-110)	2.4 (1.8-3.2)	3.6 (2.2-5.8)
<i>Chloramphenicol (orally)</i>			
None	320 (230-440)	1.7 (1.4-2.1)	1.0
A-3	120 (92-160)	1.8 (1.5-2.2)	2.7 (1.8-4.1)

\* Gamma globulin diluted with saline 1 to 4; 0.5 ml./mouse ½ hour after infection. This treatment alone was without effect.

infection, while chloramphenicol, in contrast, was active. The administration of gamma globulin along with either antibiotic did not produce a significant enhancement of protective effect (table IV).

D. pneumoniae, Strain SVI, Infection. Human gamma globulin was effective in reducing the tetracycline and chloramphenicol dosage requirements for median effect against the pneumococcal infection. The relative potency of orally administered tetracycline was 3.6 times greater for gamma globulin-treated mice than for mice without such treatment. For chloramphenicol, orally administered, the relative potency was 2.7 times greater with gamma globulin than without (table V).

Samples of three different commercial preparations of human gamma globulin (A, B, and C) were equal in their ability to reduce the tetracycline requirement for controlling a pneumococcal infection. From two to three times less antibiotic was required when used with any of these gamma globulin preparations than without (table VI). However, samples of three lots of preparation A differed in their potency with respect to producing the effect. Only two of them produced a twofold decrease in tetracycline requirement for median effect, while the third showed no action (table VII).

Gamma globulin administered intra-abdominally was more effective, in the pneumococcal infection at least, than when administered subcutaneously. In order to contain in 0.5 ml. a dose of gamma globulin that would be free of significant antipneumococcal activity when used without antibiotic, a sample of gamma globulin was diluted 1 to 4 for subcutaneous administration and at least 1 to 16

TABLE VI  
Comparison of 3 Commercial Preparations of Human Gamma Globulin for Effect  
on the ED<sub>50</sub> of Tetracycline in a Pneumococcus Infection in Mice

Gamma globulin*	Tetracycline (subcutaneously)		
	ED <sub>50</sub> , mg./Kg.	Slope function	Relative potency†
None	40 (36-45)	1.2 (1.2-1.2)	1.0
A-3	15 (13-18)	1.5 (1.3-1.7)	2.7
B-1	12 (9-17)	2.1 (1.6-2.8)	3.3
C-1	17 (13-23)	1.6 (1.3-1.9)	2.4

\* Each sample of gamma globulin diluted 1 to 4 in saline; 0.5 ml./mouse subcutaneously ½ hour after infection. This treatment alone was without effect.

† Relative potency based on ED<sub>50</sub> only, since dosage-response lines for gamma globulin-treated mice were not parallel to that of the standard (tetracycline without gamma globulin).

TABLE VII

*Comparison of 3 Lots of Gamma Globulin, Commercial Preparation A, for Effect on the ED<sub>50</sub> of Tetracycline in a Pneumococcus Infection in Mice*

Gamma globulin*	Tetracycline (subcutaneously)		
	ED <sub>50</sub> , mg./Kg.	Slope function	Relative potency
None	34 (28-42)	1.4 (1.2-1.6)	1.0
A-1	17 (13-23)	1.6 (1.4-1.9)	2.0 (1.4-2.8)
A-2	24 (18-32)	1.6 (1.3-2.0)	1.4 (1.0-2.0)
A-3	14 (10-20)	2.2 (1.5-3.5)	2.4 (1.6-3.6)

\*Each sample of gamma globulin diluted 1 to 4 with saline; 0.5 ml./mouse injected subcutaneously ½ hour after infection. This treatment alone was without effect.

for intra-abdominal administration. A 1 to 16 dilution of gamma globulin effected at least a 14-fold decrease in tetracycline ED<sub>50</sub>; 1 to 64 dilution, a twofold decrease; and a 1 to 256 dilution, no decrease (table VIII).

Gamma globulin, administered by the intra-abdominal route, was effective in modifying the ED<sub>50</sub> of tetracycline for a pneumococcal infection even when injected 24 hours before infection and subsequent treatment with the antibiotic (table IX). Administered to mice 24 hours before infection with pneumococci, it produced a 3.7-fold decrease in tetracycline ED<sub>50</sub>; six hours before infection, a 5.5-fold decrease; one hour before infection, an 11-fold decrease; and one hour after infection, an 8.3-fold decrease. The results accomplished by treatment with gamma globulin one hour before infection and one hour afterward were not significantly different.

#### DISCUSSION

The use of human gamma globulin with the tetracycline or chloramphenicol treatment of four experimental infections in mice generally resulted in a decrease of antibiotic requirement for median effect, but the decrease could not be considered statistically significant in all cases. Tetracycline and chloramphenicol were similarly affected when tested with the same sample of gamma globulin. There was variation, however, among gamma globulin samples.

Two commercial preparations of immune globulin were not significantly effective in modifying the ED<sub>50</sub> of tetracycline or chloramphenicol for a streptococcal infection. Only one of these two produced a decrease in antibiotic requirement for

TABLE VIII

*Relationship between Dosage of Gamma Globulin and Tetracycline ED<sub>50</sub> for a Pneumococcus Infection in Mice*

Gamma globulin*	Tetracycline (subcutaneously)		
	ED <sub>50</sub> , mg./Kg.	Slope function	Relative potency†
None	36 (31-42)	1.4 (1.3-1.6)	1.0
1 to 16	< 2.5		> 14.0
1 to 64	20 (15-27)	1.9 (1.5-2.4)	1.8
1 to 256	30 (25-35)	1.7 (1.5-1.9)	1.2

\* Gamma globulin (A-3) diluted in saline; 0.5 ml./mouse intra-abdominally 1 hour before infection. Alone, the 1 to 16 dilution protected 3/40 mice; 1 to 64, 0/40; 1 to 256, 0/40.

† Relative potency based on ED<sub>50</sub> only since dosage-response lines were not parallel to that of the standard (tetracycline without gamma globulin).

TABLE IX

*Effect of Time of Administration of Gamma Globulin on the ED<sub>50</sub> of Tetracycline for a Pneumococcus Infection in Mice*

Gamma globulin* time, † hr.	Tetracycline (subcutaneously)		
	ED <sub>50</sub> , mg./Kg.	Slope function	Relative potency‡
None	66 (57-77)	1.5 (1.4-1.7)	1.0
-24	18 (15-22)	1.9 (1.6-2.3)	3.7
- 6	12 ( 9-16)	3.2 (2.4-4.3)	5.5
- 1	6 ( 5- 7)	2.8 (2.3-3.4)	11.0
+ 1	8 ( 6-10)	2.3 (1.8-2.9)	8.3

\* Gamma globulin (A-4) diluted in saline 1 to 20; 0.5 ml./mouse intra-abdominally. Treatment with gamma globulin alone was without significant effect.

† Time of administration relative to time of infection.

‡ Relative potency based on ED<sub>50</sub> only since dosage-response lines were not parallel to that of the standard (tetracycline without gamma globulin).

control of a staphylococcal infection. Yet both of these preparations were equally effective with respect to decreasing the tetracycline or chloramphenicol ED<sub>50</sub> in the pneumococcal infection. Against the latter infection, three different commercial samples produced similar beneficial results, but one of three lots of a commercial preparation did not.

The variations cannot be readily explained. Fisher and Manning<sup>6</sup> as well as Reedy et al<sup>7</sup> have suggested that the specific antibody is the factor in gamma globulin that is largely or entirely responsible for antibacterial action in experimental infections. Commercially prepared immune globulin is a solution of  $16 \pm 1.5$  per cent gamma globulin, which meets certain requirements for poliomyelitis and measles antibody content. Since each lot is prepared from large pools of blood or placenta from many donors, one would expect, generally, the content of a particular antibody to be relatively similar in various lots. In fact, Heyl et al<sup>8</sup> found that human gamma globulin derived from donors in geographically different sections of the United States contained relatively equal amounts of neutralizing antibody against the virus of herpes simplex. However, even slight variations in kind and quantity of bacterial antibodies, which probably exist among several lots of immune globulin, may be sufficient to affect the results of tests such as are described here.

Although our results with gamma globulin and chloramphenicol do not appear to be as striking as those reported elsewhere,<sup>1</sup> they do provide additional support to the observation that gamma globulin-antibiotic combinations can be more effective than the antibiotics alone in controlling experimental infections.

#### SUMMARY

The use of human gamma globulin in conjunction with tetracycline or chloramphenicol reduced to a significant degree the amount of antibiotic needed for a median effect against *Staph. aureus*, strain Smith, and *D. pneumoniae*, strain SV1, infections in mice. Not all lots of commercially prepared gamma globulin were effective.

Samples of human gamma globulin active in these infections were not effective with either antibiotic against *Str. pyogenes*, strain C203, or *Staph. aureus*, strain Rose, infections.

Gamma globulin was more effective when administered by the intra-abdominal

route than by the subcutaneous route. The beneficial effects of gamma globulin injected intra-abdominally were evident even when it was administered 24 hours before infection.

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#### BIBLIOGRAPHY

1. FISHER, M. W.: Synergism between human gamma globulin and chloramphenicol in the treatment of experimental bacterial infections, *Antib. & Chemo.* 7:315, 1957.
2. MANNING, M. C.; GAGLIARDI, L. A., AND FISHER, M. W.: The therapeutic activity of gamma globulin—chloramphenicol combinations in mouse infections due to *Salmonella*, pneumococci, *Escherichia coli*, or *Pasteurella*. In: *Antibiotics Annual 1957–1958*, New York, Medical Encyclopedia, Inc., 1958, p. 566.
3. HOLPER, J. C.; RICKHER, C. J., AND SYLVESTER, J. C.: Ristocetin: effect of gamma globulin on in vivo activity. In: *Antibiotics Annual 1957–1958*, New York, Medical Encyclopedia, Inc., 1958, p. 577.
4. WAISBREN, B. A.: The treatment of bacterial infections with the combination of antibiotics and gamma globulin, *Antib. & Chemo.* 7:322, 1957.
5. KNOUF, E. G.: The combined use of gamma globulin and chloramphenicol in the treatment of refractory infections: a report of ten cases. In: *Antibiotics Annual 1957–1958*, New York, Medical Encyclopedia, Inc., 1958, p. 584.
6. FISHER, M. W., AND MANNING, M. C.: The specific antibody nature of the therapeutic action of gamma globulin in experimental bacterial infections in mice. In: *Antibiotics Annual 1957–1958*, New York, Medical Encyclopedia, Inc., 1958, p. 572.
7. REEDY, R. J.; OSWALD, E. J., AND WRIGHT, W. W.: The effect of gamma globulin and specific antiserum combined with antibiotics in experimental infections in mice. In: *Antibiotics Annual 1957–1958*, New York, Medical Encyclopedia, Inc., 1958, p. 581.
8. HEYL, J. T.; ALLEN, H. F., AND CHEEVER, F. S.: Quantitative assay of neutralizing antibody content of pools of gamma globulin from different sections of the United States against the viruses of herpes simplex, lymphocytic choriomeningitis and epidemic keratoconjunctivitis, *J. Immunol.* 60:37–45, 1948.

# The Influence of Submaximal Antibiotic Levels on the Growth of Chlortetracycline-Resistant Bacteria

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During an investigation of means of controlling antibiotic-resistant bacteria,<sup>1</sup> it was observed that submaximal levels of chlortetracycline, defined as less than the minimal inhibitory concentration, profoundly affected the growth of chlortetracycline-resistant staphylococci. This phenomenon had been previously observed in the case of isoniazid<sup>2</sup> and *p*-aminosalicylic acid resistant<sup>3</sup> strains of *Mycobacterium tuberculosis*, and it has recently been reported to occur in a chloramphenicol-resistant strain of *Staphylococcus aureus*.<sup>4</sup> Extensive effort has been expended in the study of the origins of drug resistance, but comparatively few studies have been made of physiological variations produced in a drug-resistant organism by growth in the presence of the drug.<sup>5</sup> We have therefore investigated the behavior of chlortetracycline-resistant organisms in the presence of submaximal concentrations of antibiotics and have studied a number of factors which affect the observed level of resistance of a cell.

## MATERIALS AND METHODS

**Bacterial Strains.** *Staphylococcus albus* 69R (*Micrococcus pyogenes* var. *albus* 69R), capable of growth in at least 125  $\gamma$ /ml. chlortetracycline, was used as a resistant test strain. *Staph. aureus* 209P (*M. pyogenes* var. *aureus* 209P) was used as a chlortetracycline-sensitive test organism. Two strains of chlortetracycline-resistant psychrophilic pseudomonads, *Pseudomonas* sp. R42 and *Pseudomonas* sp. B235, were also used. All cultures were obtained from stock collections in these laboratories.

**Experimental Methods.** Stock cultures were maintained on Trypticase soy agar slants. Parallel stock cultures of *Staph. albus* 69R were maintained in the presence and absence of 50  $\gamma$ /ml. chlortetracycline. Turbidimetric growth curve experiments were performed in Trypticase soy broth adjusted to pH 7.3 or 6.5. At least two replicate tubes were run for each experimental condition. The medium was pre-incubated at the experimental growth temperature to avoid temperature shock during transfer of inocula. Inocula consisted of 0.1 or 0.2 ml. of a six hour (late exponential phase) or 18 hour broth cultures. These were added to tubes containing 5.0 ml. broth. Antibiotic solutions and/or distilled water were added to give a final volume of 6.1 or 6.2 ml./tube. The staphylococci were incubated at 37 C. The pseudomonads were grown at 28 and 4 C. Optical density measurements were made on a Coleman Model 11 spectrophotometer at a wave length of 610 m $\mu$ .

## RESULTS

The effect of varying concentrations of chlortetracycline on the growth of *Staph. albus* 69R is illustrated in figure 1. The principal effect was a prolongation of the lag and acceleration phases of growth, which was sometimes accompanied by a slight decrease in exponential growth rate and maximum population density. Cells

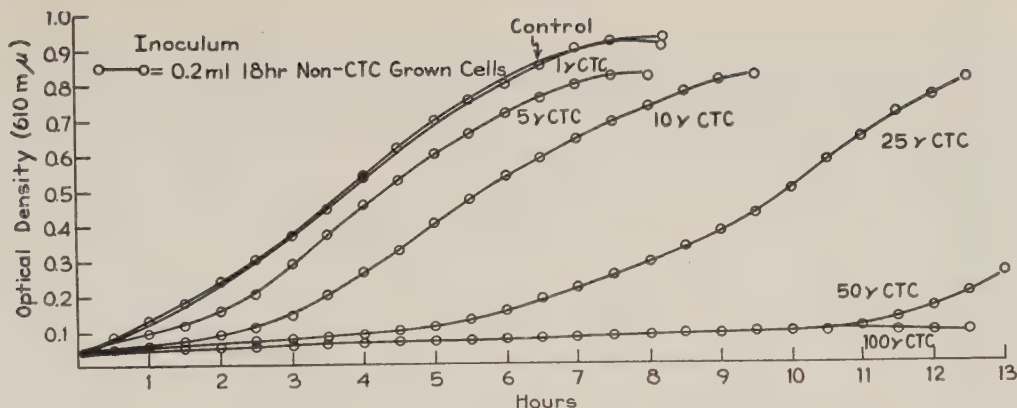


FIG. 1. The effect of chlortetracycline on the growth of *Staphylococcus albus* 69R is shown. CTC, chlortetracycline.

exposed to 100  $\gamma$ /ml. had not yet begun to divide at 13 hours but did reach maximum turbidity within 22 hours at 37 C. Chlortetracycline-induced lags varied with the physiological age and size of the inoculum. A hundredfold decrease in the size of the inoculum resulted in an increased lag in both control and antibiotic grown cells (fig. 2). The lag effect could be drastically altered by exposing the inoculum to chlortetracycline prior to the experiment. Figure 3 shows the effect of 25  $\gamma$ /ml. chlortetracycline on cells subcultured in the presence and absence of chlortetracycline prior to the experiment. Inhibition of chlortetracycline-grown cells was markedly less than that shown by the non-chlortetracycline grown inoculum. Continued transfer for 10 or more subcultures, in a medium containing 50  $\gamma$ /ml. chlortetracycline, reduced the growth lag produced by submaximal chlortetracycline levels but did not abolish it. If a chlortetracycline-grown culture of *Staph. albus* 69R was carried for one subculture in the absence of chlortetracycline and then re-inoculated to broth containing the drug, the resultant lag in growth approximated that shown by an inoculum of non-chlortetracycline grown cells (fig. 4). The rapidity with which this reversion took place suggests that it is an adaptive phenomenon, perhaps akin to the diluting out of an induced enzyme when induced cells are grown in the absence of substrate. For purposes of comparison, *Staph. aureus* 209P, a chlortetracycline-sensitive organism, was tested. Growth of this strain in the presence of submaximal levels of chlortetracycline resulted in a marked decrease in exponential growth rate rather than long extension of the lag phase. This is in contrast to the

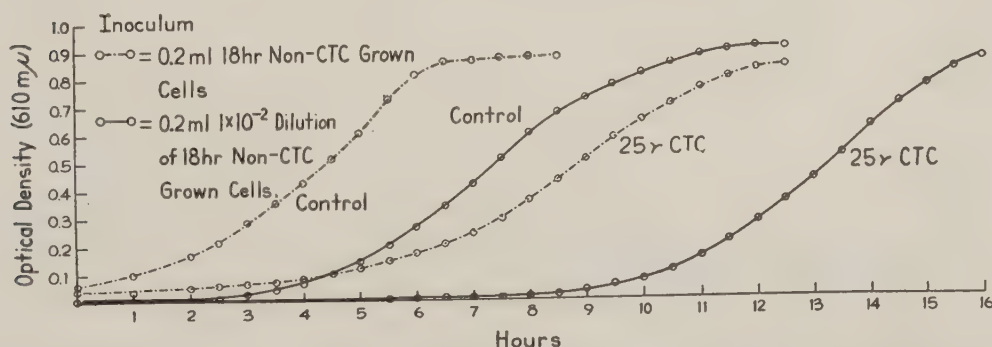


FIG. 2. The effect of the size of the inoculum on the response of *Staphylococcus albus* 69R to chlortetracycline is illustrated. CTC, chlortetracycline.

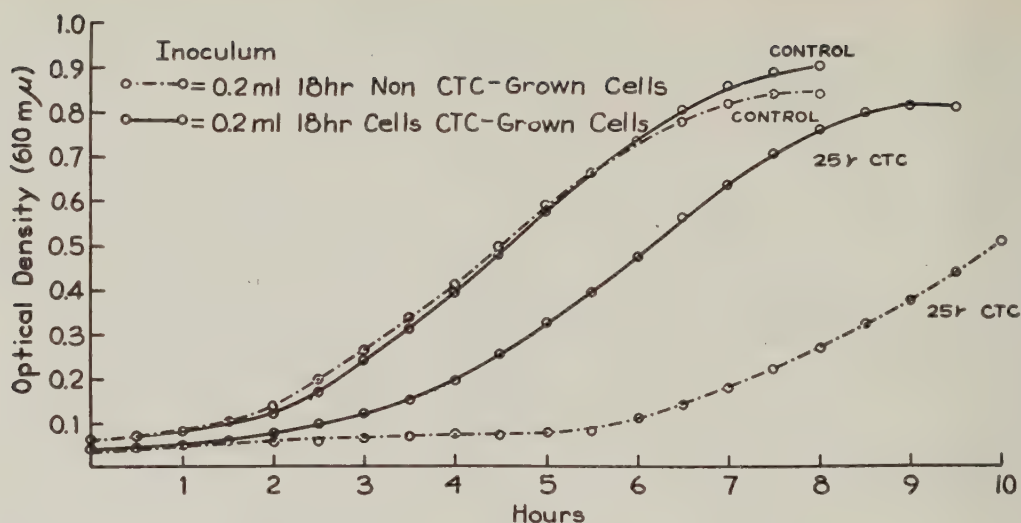


FIG. 3. The effect of prior exposure to chlortetracycline on the response of *Staphylococcus albus* 69R to chlortetracycline is shown. CTC, chlortetracycline.

results obtained with *Staph. albus* 69R. Continued transfer of the sensitive strain on a chlortetracycline-containing medium resulted in some decrease in the inhibitory effect of a submaximal drug level (table I).

The effect of incubation temperature in altering the magnitude of the lag phenomenon was strikingly demonstrated in the case of chlortetracycline-resistant psychrophilic pseudomonads. These organisms will grow optimally at 25 to 28 C., more slowly at 4 C., but not at all at 37 C. Figure 5 illustrates the response of *Pseudomonas* sp. B235 to chlortetracycline at 28 and 4 C. At the higher temperature 25  $\gamma$ /ml. chlortetracycline barely affected the growth rate. At 4 C., as little as 5  $\gamma$ /ml. chlortetracycline caused a significant increase in the lag phase and a decrease in the exponential growth rate of *Pseudomonas* sp. B235. Ramsey<sup>6</sup> has reported that growth at a temperature other than optimal results in decreased resistance to anti-

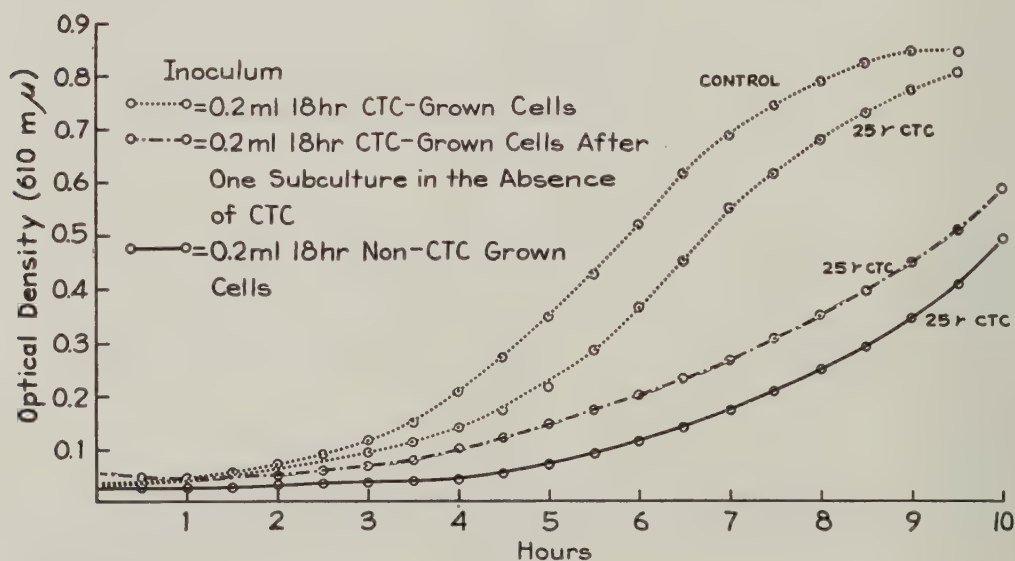


FIG. 4. The response of chlortetracycline-grown cells to chlortetracycline after a single subculture in the absence of the drug is illustrated. CTC, chlortetracycline.

TABLE I

Response to Submaximal Levels of Chlortetracycline by *Staph. aureus* 209P Maintained in the Presence and Absence of 0.25  $\gamma$ /ml. Chlortetracycline

	Concentration of chlortetracycline in medium, $\gamma$ /ml.	Time of incubation, hours	Optical density (610 $m\mu$ )
Non-chlortetracycline grown cells	0	2	0.452
	0	6	1.124
	0.0625	2	0.180
	0.0625	6	0.428
	0.25	2	0.1135
	0.25	6	0.176
Chlortetracycline grown cells	0	2	0.374
	0	6	0.949
	0.0625	2	0.351
	0.0625	6	0.921
	0.25	2	0.213
	0.25	6	0.515

biotics. At low temperatures, chlortetracycline delayed growth of resistant *Pseudomonas* by a matter of days rather than hours. This can be of importance in the problem of food spoilage. In figure 6, *Pseudomonas* sp. R42, a chlortetracycline-resistant food spoilage organism, was partially inhibited by 10  $\gamma$ /ml. chlortetracycline at 4 C. This organism was also partially inhibited by 400  $\gamma$ /ml. of tetrasodium ethylenediaminetetraacetic acid (EDTA) at 4 C. and pH 6.5. A marked enhancement of inhibition was noted when the resistant strain was treated with a combination of 10  $\gamma$ /ml. chlortetracycline and 400  $\gamma$ /ml. EDTA. The growth lag resulting from this treatment was at least 38 days.

## DISCUSSION

*Staph. albus* 69R was profoundly affected by concentrations of chlortetracycline far lower than the so-called "minimum inhibitory concentration." The primary effect was a pronounced increase in the lag and acceleration growth phases. The exponential growth rate and maximum population density were only slightly affected. The relative magnitude of these effects varied with the physiological age of the inoculum, size of the inoculum, level of chlortetracycline employed, and prior ex-

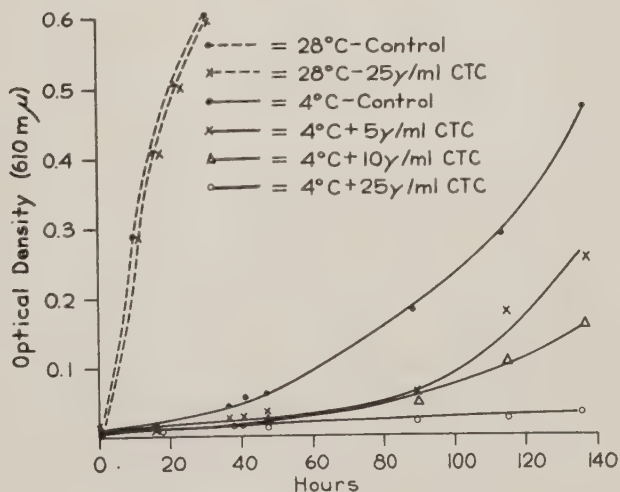


FIG. 5. The influence of temperature on chlortetracycline resistance of *Pseudomonas* sp. B235 is indicated. CTC, chlortetracycline.

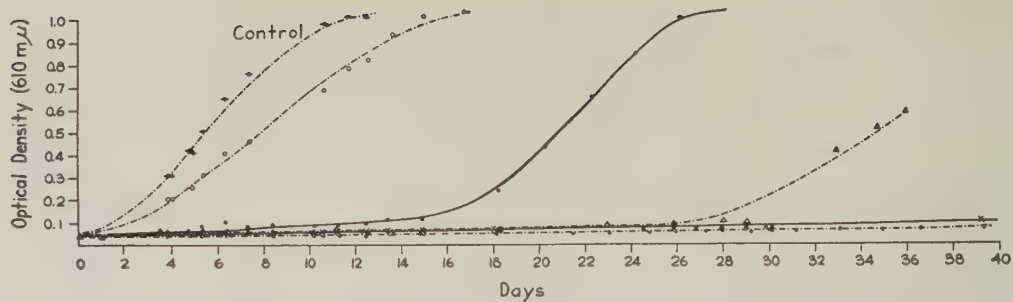


FIG. 6. The effect of EDTA and chlortetracycline on the growth of *Pseudomonas* sp. R42 at pH 6.5 and 4 C. •—•—• control; o—o—o 10 $\gamma$ /ml. chlortetracycline; •—•—• 400 $\gamma$ /ml. EDTA;  $\Delta$ — $\Delta$ — $\Delta$  500 $\gamma$ /ml. EDTA; X—X—X 400 $\gamma$ /ml. EDTA + 10 $\gamma$ /ml. chlortetracycline; •—•—• 500 $\gamma$ /ml. EDTA + 10 $\gamma$ /ml. chlortetracycline.

posure or nonexposure of the inoculum to chlortetracycline. Growth of a chlortetracycline-sensitive strain, *Staph. aureus* 209P, in submaximal drug levels resulted primarily in a decrease in exponential growth rate, with a less marked increase in the lag and acceleration phases of growth. The composition of the medium and degree of aerobiosis, factors that might exert considerable influence on the response of an organism to antibiotics, were kept constant throughout the experiments. The response to chlortetracycline of individual cells within a given microbial population may differ from that of the population as a whole. This fact must be borne in mind in any interpretation of the turbidimetric growth curve data reported here.

On a population level, one explanation for the results obtained with *Staph. albus* 69R is that the proportion of cells in the inoculum competent for cell division decreases as the level of chlortetracycline increases. This would not be unexpected in a wild type population, such as a culture of *Staph. aureus* 209P, which might contain only a minute percentage of resistant cells. Indeed, the gradual development of a few mutants within an initially sensitive population was probably responsible for the growth obtained with the sensitive strain. *Staph. albus* 69R does not represent a wild type population. It was selected from an environment containing 125  $\gamma$ /ml. chlortetracycline and consistently attained maximum growth, within 24 hours, in Trypticase soy medium containing 50  $\gamma$ /ml. chlortetracycline. This suggests that the vast majority of cells in an *Staph. albus* 69R culture were highly chlortetracycline resistant. It does not guarantee that such a population will not partially revert, by mutation, to chlortetracycline sensitivity if it is maintained in the absence of the drug. In order to mitigate against this possibility, cells were routinely carried on Trypticase soy medium containing 50  $\gamma$ /ml. chlortetracycline. The presence of chlortetracycline in the medium provided a barrier against the development of sensitive or at least less-resistant cells within the population.

After 10 or more consecutive transfers in such an environment, *Staph. albus* 69R still exhibited a measureable growth lag upon subsequent inoculation to medium containing 25 or 50  $\gamma$ /ml. chlortetracycline. It is reasonable to assume that such an inoculum is wholly or almost wholly composed of highly resistant cells. One can postulate that inhibition of growth observed under these conditions is due to the deleterious effect of chlortetracycline on the population as a whole rather than a reflection of the gradual development of a few cells within the population. These experiments do not rule out the mechanism of selection; they do, however, minimize its influence.

The possibility that the lag phenomenon is merely a reflection of the gradual

inactivation of chlortetracycline in the medium must be considered. This explanation seems unlikely for several reasons. Tetracycline is more stable in solution than chlortetracycline, yet it affects *Staph. albus* 69R in a manner similar to that of chlortetracycline. A change in pH of the medium from 7.3 to 6.5 has little effect on the growth of *Staph. albus* 69R in the presence or absence of chlortetracycline but does significantly retard breakdown of the antibiotic. Finally, if the lag effect were dependent upon the inactivation of chlortetracycline, a plot of the logarithm of chlortetracycline concentration versus the time of growth initiation at each drug level would give a straight line; our data plotted in this manner do not give a straight line. The response of chlortetracycline-grown *Staph. albus* 69R to the antibiotic nearly parallels that of non-chlortetracycline grown cells after a single subculture in the absence of the drug (fig. 4). This effect might be attributed to a reversion of the culture to partial sensitivity similar to that observed by Hughes<sup>7</sup> in the case of a penicillin-resistant *Staphylococcus*. However, an increase in lag of the magnitude shown in figure 4 corresponds roughly to that obtained by diluting the inoculum one hundredfold. It seems unlikely that greater than 90 per cent of the population would revert in so short a time. If only a few cells mutated to sensitivity, they would have to possess a fantastic advantage in growth rate over the remainder of the population to account for the results obtained. Therefore, a more likely interpretation of this phenomenon would be that it is an adaptive response. That is, resistant cells of the same genotype can exhibit different phenotypic responses to submaximal antibiotic levels depending upon their "biochemical history." The genetic constitution of *Staph. albus* 69R enables it to grow at a given level of chlortetracycline. The rate at which it does this is subject to variations in the environment. The maximum rate of growth in submaximal drug levels is attained only after continued subculture in the presence of chlortetracycline. The ability to maintain this maximum rate is lost fairly rapidly when the cells are subcultured in the absence of chlortetracycline.

Although the data suggest that *Staph. albus* 69R adapts to submaximal drug levels, they leave unanswered the vital question as to what metabolic systems may be involved in this process. The adaptive response might be due to the formation of altered enzymes, increased amounts of enzymes already present, changes in a permeation mechanism, or a combination of these.

Psychrophilic pseudomonads are admirable models for the demonstration of the influence of temperature on the phenotypic response of resistant cells. As shown in figure 5, a reduction in incubation temperature from 28 to 4 C. markedly reduced the observed chlortetracycline resistance of *Pseudomonas* sp. B235. It is possible that a reduction in temperature increases the sensitivity of a given metabolic system to chlortetracycline. The increase in stability of chlortetracycline at low temperatures is insufficient to account for the results obtained.

A number of reports<sup>8</sup> have suggested that the antibiotic activity of tetracycline antibiotics is related to their chelating ability. Therefore, a combination of chlortetracycline with a strong chelating agent, EDTA, which has been reported to enhance the activity of tetracycline antibiotics in milk,<sup>9</sup> was employed against the psychrophils in an attempt to prolong the chlortetracycline-induced lag indefinitely. EDTA was found to be inhibitory per se at relatively high levels, and to be synergistic with chlortetracycline (fig. 6). The effectiveness of EDTA was found to depend upon the pH of the medium: It was considerably more effective, on a weight basis, at pH 6.5 than at 7.3 both alone and in the presence of chlortetracycline. Deatherage et al<sup>10</sup> have shown that chlortetracycline is more effective on whole meat than in broth against chlortetracycline-insensitive beef spoilage organisms. In this case,

whole meat may be merely a less efficient medium than culture broth, thus diminishing the ability of resistant strains to grow in submaximal antibiotic concentrations. The observations of Deatherage and his co-workers may be still another example of the influence of environmental variation on the phenotypic response of resistant organisms to chlortetracycline.

#### SUMMARY

The response of resistant organisms to submaximal levels of chlortetracycline has been evaluated. Submaximal drug levels markedly inhibited growth of resistant staphylococci and psychrophilic pseudomonads. This inhibitory effect was manifested as an extension of the lag and acceleration growth phases, accompanied by some decrease in the exponential growth rate and maximum population density. The inhibitory effect appears to be a phenotypic response. The degree to which it is expressed is subject to variations in the environment such as alterations in the size and age of inoculum, temperature of incubation, and prior exposure or nonexposure of the cultures to chlortetracycline. Continued exposure to chlortetracycline enables a highly resistant strain of *Staphylococcus*, *Staphylococcus albus* 69R, to attain a maximal rate of growth in submaximal concentrations of chlortetracycline. After a single subculture in the absence of chlortetracycline followed by re-introduction to medium containing chlortetracycline, the growth curve of the culture approximates that obtained with a population of *Staph. albus* 69R, which has been continuously maintained in the absence of chlortetracycline. This phenomenon may be an example of phenotypic adaptation to chlortetracycline by a genotypically resistant organism.

The influence of temperature on the resistance process was strikingly demonstrated in the case of two resistant strains of psychrophilic pseudomonads, *Pseudomonas* sp. R42 and B235. Both organisms were little affected by 10  $\gamma$ /ml. chlortetracycline when grown at 28 C. At 4 C., 10  $\gamma$ /ml. chlortetracycline profoundly inhibited growth. The inhibitory activity of chlortetracycline against the psychrophils was enhanced by combination with the chelating agent, ethylenediaminetetraacetic acid.

#### BIBLIOGRAPHY

1. HALL, R. H., AND GALE, G. O.: Augmentation of chlortetracycline activity against an antibiotic resistant strain of *Micrococcus pyogenes* var. *albus* by kinetin and other 6-(substituted)aminopurines. In press.
2. PEIZER, L. R.; WIDELock, D., AND KLEIN, S.: Effect of isoniazid on the viability of isoniazid-susceptible and isoniazid-resistant cultures of *Mycobacterium tuberculosis*, *Am. Rev. Tuberc.* 69:1022, 1954.
3. TSUKAMURA, M.: The properties of para-aminosalicylic acid-resistant strains of *Mycobacterium tuberculosis* var. *hominis*, *Am. Rev. Tuberc.* 75:608, 1957.
4. RAMSEY, H. H.: The growth of sensitive and resistant strains of *Micrococcus pyogenes* in the presence of varying concentrations of chloramphenicol, *Antib. & Chemo.* 8:250, 1958.
5. DAVIS, B. D.: Ciba Foundation Symposium on Drug Resistance in Microorganisms, Boston, Little, Brown and Company, 1957, p. 169.
6. RAMSEY, H. H.: Influence of temperature on inhibition of *Micrococcus pyogenes* by chloramphenicol, *J. Bact.* 73:689, 1957.
7. HUGHES, W. H.: Variations in penicillin resistance in single cell cultures of *Staphylococcus aureus*, *J. Gen. Microbiol.* 6:175, 1952.
8. WEINBERG, E. D.: The mutual effects of antimicrobial compounds and metallic cations, *Bact. Rev.* 21:46, 1957.
9. PRICE, K. E.; ZOLLI, Z., JR.; ATKINSON, J. C., AND LUTHER, H. G.: Antibiotic inhibitors I: The effect of certain milk constituents, *Antib. & Chemo.* 7:672, 1957.
10. DEATHERAGE, F. E.; JAY, J. M., AND WEISER, H. H.: Mode of action of chlortetracycline in controlling meat spoilage. Abst. 132nd Meet. Am. Chem. Soc. 26A, 1957.

# Palatability of Broad-Spectrum Antibiotics for Swine

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Certain antibiotics are known to have growth-promoting properties when fed to livestock. Among recent reviews of this action in swine are those by Cunha<sup>1</sup> and Luther and Reynolds.<sup>2</sup> Investigators at the Florida Agricultural Experiment Station<sup>3,4</sup> have reported that feed consumption by swine varied according to the type of antibiotic, when the animals had a choice. The Florida investigators found that feeds containing erythromycin were consumed scarcely at all when feeds without this antibiotic were available. They stated that feeds containing chlortetracycline,<sup>†</sup> penicillin, or oxytetracycline<sup>‡</sup> were consumed in that order of preference.

Inasmuch as the Florida work was conducted with crystalline antibiotics only, it was of interest to compare palatability of feeds into which the antibiotics had been incorporated by the use of commercially available antibiotic feed supplements. It was further of interest to study the growth of pigs on feeds containing different antibiotics when no choice was allowed.

## METHODS

*Experiment 1.* This paralleled the Florida work, in that comparative feed consumption was determined when the antibiotics were added in crystalline form and the animals had a choice of feed. A basal full ration feed of composition shown in table I was fed to pigs of 70 lb. average starting weight. In two replicated pens of 28 pigs each, one hopper offered this ration supplemented with crystalline oxytetracycline, 40 Gm./ton, and another hopper offered this ration supplemented with a like quantity of crystalline chlortetracycline. Positions of these hoppers were interchanged daily to eliminate a possible location preference. Consumption of feed was recorded weekly and the trial was terminated at eight weeks when the pigs had attained an average weight of 160 lb. The same procedure was applied to another pair of replicates of 6 pigs each.

*Experiment 2.* This was a comparison of relative feed intake when antibiotics were supplied by commercial feed supplements, rather than in crystalline form. The basal ration, also shown in table I, was fed to replicated pens of 6 pigs of 37 lb. average starting weight. This ration supplemented with Aurolac-10<sup>§</sup> was offered in one hopper and the same ration supplemented with TM-10<sup>||</sup> was offered in a second hopper. Antibiotic level was equivalent to 40 Gm./ton. Position of hoppers was interchanged daily. The quantities of these respective supplements included per ton of feed were equal. Consumption of feed was recorded weekly and the trial was terminated when the average weight of the pigs was 210 lb.

*Experiment 3.* This was a conventional growth trial to compare rate of gain and

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† The trade name of Lederle Laboratories Division, American Cyanamid Company, for chlortetracycline is Aureomycin.

‡ The trade name of Chas. Pfizer & Co. for oxytetracycline is Terramycin.

§ Antibiotic feed supplement, Lederle Laboratories Division, American Cyanamid Co.

|| Antibiotic feed supplement, Chas. Pfizer & Co., Inc.

TABLE I  
Composition of Basal Ration

	Experiment 1		Experiments 2 and 3	
	Start to 125 lb., %	125 lb. to end, %	Start to 125 lb., %	125 lb. to end, %
Ground yellow corn	51.54	49.83	73.10	83.10
Ground oats	23.71	30.83	—	—
44% soybean oil meal	—	—	18.00	9.00
50% soybean oil meal	15.85	10.44	—	—
50% meat and bone scraps	1.50	1.50	2.00	1.00
60% tankage	2.50	2.50	—	—
Dehydrated alfalfa meal	2.50	2.50	5.00	5.00
Steamed bonemeal	1.00	1.00	0.50	0.50
Ground limestone	0.50	0.50	0.50	0.50
Iodized salt	0.50	0.50	0.50	0.50
Trace mineral mix*	0.05	0.05	0.05	0.05
Vitamin mix†	0.35	0.35	0.35	0.35
	100.00	100.00	100.00	100.00

\* The trace mineral mix contributed Fe, Cu, Co, Mn, and Zn.

† The vitamin mix contributed vitamin A, vitamin D, riboflavin, niacin, pantothenic acid, choline, vitamin B<sub>12</sub>, and methionine.

feed efficiency of pigs fed rations supplemented with either crystalline oxytetracycline or crystalline chlortetracycline but allowed no choice between the two. The basal ration was the same as that used in experiment 2. There were two replicates of 6 pigs each for control, oxytetracycline and chlortetracycline treatments, respectively, antibiotics being supplied at 40 Gm./ton. The trial extended until the supplemented pigs attained 210 lb.

#### RESULTS AND DISCUSSION

Table II shows that, in three of four replications, the pigs allowed a choice consumed more of the ration containing crystalline chlortetracycline. This is in agreement with the findings of Tomlin et al.<sup>3</sup> However, when the antibiotics were added to feed in the customary commercial carriers, the animals chose to consume more of the ration containing oxytetracycline, as shown in table III.

It is evident from these findings that differences in ration palatability apparently resulting from the addition of certain crystalline antibiotics do not necessarily apply when premixes or feed supplements are used as the source of these antibiotics in feeds.

TABLE II  
Effect of Crystalline Antibiotics on Feed Consumption

Replicate	No. pigs	Total feed consumed, lb.	
		Oxytetracycline hydrochloride 40 Gm./ton	Chlortetracycline hydrochloride 40 Gm./ton
1	28	3327	5317
2	28	3934	4940
3	6	954	2925
4	6	2375	1648
Total		10,590	14,830
Consumption ratio		42%	58%

TABLE III

*Effect of Commercial Antibiotic Feed Supplements Upon Feed Consumption*

Replicate	No. pigs	Total feed consumed, lb.	
		Oxytetracycline,* 40 Gm./ton	Chlortetracycline,* 40 Gm./ton
1	6	2754	932
2	6	2668	726
Total		5422	1658
Consumption ratio		77%	23%

\* Supplied as the antibiotic feed supplement.

For the growth trial, where the animals had no choice between antibiotic sources, table IV shows no noteworthy differences in performance, even though crystalline antibiotics, rather than commercial feed supplement products, were used. Only insignificant differences in feed consumption and feed efficiency were obtained between the two antibiotics. Both oxytetracycline and chlortetracycline improved rate of gain and feed efficiency over that on the unsupplemented basal ration. Thus, although a difference in palatability or other uncontrollable factors may be expressed as a wide consumption ratio in preference studies where swine have a choice, it may have no consequence when they have no choice.

## SUMMARY

Growing finishing pigs were found to consume more of a ration containing 40 Gm./ton of oxytetracycline added as a feed supplement than of a feed fortified in a similar manner with 40 Gm./ton of chlortetracycline, although the opposite was found when these antibiotics were supplied in pure crystalline form.

TABLE IV

*Comparison of Crystalline Oxytetracycline and Chlortetracycline as Growth Promotants*

	Control	Oxytetracycline hydrochloride 40 Gm./ton	Chlortetracycline hydrochloride, 40 Gm./ton
No. pigs	2x6	2x6	2x6
Feed Consumption, lb.			
Replicate 1	3796	3736	3729
Replicate 2	4267*	3452	3667
Average	4032*	3594	3698
Daily Gain, lb.			
Replicate 1	1.54	1.53	1.61
Replicate 2	1.41	1.59	1.61
Average	1.48	1.56	1.61
Lb. feed/lb. gain			
Replicate 1	3.67	3.61	3.61
Replicate 2	4.11	3.31	3.51
Average	3.89	3.46	3.56

\* No direct comparison with consumption of the supplemented feeds can be made because of slower growth and thereby prolonged growing time. Treatments were terminated at approximately equal weight.

When pigs were not permitted a choice, they consumed essentially equal amounts of rations fortified with the respective crystalline antibiotics and no difference was found between the two antibiotics with respect to growth stimulation and improvement of feed efficiency.

#### BIBLIOGRAPHY

1. CUNHA, T. J.: Antibiotics for swine, beef cattle, sheep and dairy cattle. Proc. First Int. Conf. on Use of Antibiotics in Agriculture, Pub. 397, National Academy of Science-National Research Council, Washington, D. C., 1956, p. 9.
2. LUTHER, H. G., AND REYNOLDS, W. M.: Low-level antibiotics for swine. Industrial Application of Antibiotics in Agriculture, Symposium Series No. 2, Chas. Pfizer & Co., Inc., 1956, p. 15.
3. TOMLIN, D. C.; WALLACE, H. D.; COMBS, G. E., JR., AND ALSMEYER, S. L.: Studies on the palatability of antibiotics for swine, *J. Animal Sc.* 15:1230, 1956.
4. TOMLIN, D. C.; WALLACE, H. D., AND COMBS, G. E., JR.: The influence of certain antibiotics on the palatability of swine rations, *J. Animal Sc.* 17:42, 1958.

# The Current Status of Erythromycin, Kanamycin, Novobiocin, Oleandomycin, Ristocetin, and Vancomycin, with Particular Reference to Their Use in Staphylococcal Disease

## Panel Discussion

Maxwell Finland, Moderator

### Members of the Panel

E. L. Foltz  
J. E. Geraci  
W. M. M. Kirby

E. L. Quinn  
M. J. Romansky  
E. M. Yow

**Dr. Finland** (Moderator): Ladies and gentlemen, welcome to this panel discussion on the timely status of a number of antibiotics that are presumably useful in the treatment of disease, particularly in staphylococcal disease.

There are six antibiotics mentioned in the published title of this panel and we have asked each one of the members of the panel to take up one of them and summarize its present status. However, it is not meant for this discussion to be limited to these six antibiotics alone, because we should have opportunities to get into discussions about other antibiotics in relation to these.

In order to get a sort of orientation after the many papers that have been presented on the subject matter of this panel, and some that are still to be presented in future meetings, I have asked each one of the members of the panel to discuss the present status, in his opinion, of one particular antibiotic, but before doing so, I'm going to introduce the members of the panel. First is Dr. Foltz, who is Assistant Professor of Medicine at the University of Pennsylvania, and who like all of the individuals in this panel, is what we might call a specialist in infectious diseases, who has had experience, and is having a continuing experience in the field of infectious diseases and chemotherapy, both in the laboratory and in the clinic. The second member of the panel is Dr. Geraci, who is Assistant Professor of Medicine in the Graduate School of the University of Minnesota and Consultant in Medicine at the Mayo Clinic. On my immediate right is Dr. Kirby, Professor of Medicine in the University of Washington at Seattle. On my immediate left, Dr. Quinn, who is Physician in Charge of the Infectious Diseases Clinic at the Henry Ford Hospital, Detroit. To his left, Dr. Romansky, Professor of Medicine at George Washington University here in Washington. Last is Dr. Ellard Yow, Professor of Medicine at Baylor University Medical School in Houston, Texas. I am going to call these men in alphabetical order without reference to the antibiotics they discuss, so that we thus achieve a sort of random distribution of these agents. I am going to ask each panel member to make it very brief so that we can have a few rounds of discussion on some very important questions. I must say that they have not been rehearsed, at least I do not know what anybody is going to say, and I do not think that any one knows what the other one is going to say. We will hear first from Dr. Foltz, who will talk to us about the present status, in his opinion, of novobiocin.

**Dr. Foltz:** Thank you, Dr. Finland. I think there are two functions that we as members of the panel should assume this afternoon. First, to review what has been recorded in the literature about these drugs, as far as their clinical effectiveness is concerned, and then to give, secondly, some of our own experiences. I have attempted to review those papers that reported the treatment of staphylococcal infections with novobiocin, and that recorded the success of therapy. Since 1955 I found at least 28 papers in which more than 500 cases of staphylococcal infection have been treated. Of these 503 cases, 373 patients

had satisfactory or better clinical responses. That gives us then an approximate satisfactory response rate of 74 per cent. This morning, Dr. Greenberg presented a paper of 130 consecutive surgical cases treated with novobiocin, of which there were at least 100 who had good responses or better with staphylococcal infections proved in bacteriological studies. There was a satisfactory response of 83 per cent in this group.

It appears in the literature that novobiocin should be effective and useful in the management of such diseases as lobar pneumonia, bronchial pneumonia, and severe respiratory infections that are accompanied with invasion by the staphylococci. It is effective in soft-tissue infections, including abscesses, furuncles, wound infections, and other localized infection. It has been found useful in urinary tract infections. The poor responses in the literature have generally been associated with septicemias, severe pneumonias, particularly those that are complicating other basic diseases, and with surgical infections in which the drainage was difficult either to initiate or maintain for any period of time.

Now, I would like to mention briefly some of our experiences at the Hospital of the University of Pennsylvania. First of all, let me say novobiocin has never been used promiscuously at our institution, and in recent months we have even cautioned our staff to use it only in severe infections. However, as you know, surgeons cannot be completely intimidated and, since most of our staphylococcal infections are in the surgical group, we do find that it has been used, and with a certain degree of success. It has been recommended primarily for use in the treatment of hospital acquired staphylococcal infections or in moderately severe infections that have been brought in from the outside. It has not been recommended in the management of the uncomplicated furuncle. It is encouraging in the series that I have reviewed to find that at least 20 to 25 per cent of the cases have been treated with no antibiotics whatsoever. Novobiocin is not a substitute for surgical drainage when infection is a surgical problem.

We have utilized novobiocin primarily in recent months in combination with chloramphenicol. The reason for this has been based on the theoretical assumption that a resistant mutant is less likely to emerge resistant to two antibiotics when those two antibiotics are given concurrently. In the past four months we have had 20 cases in which novobiocin was apparently the keystone of treatment as far as the antistaphylococcal therapy is concerned. We had 16 cases in which the results were good, 1 in which the result was poor, and 3 failures. Two patients with bronchial pneumonia responded very well; 13 cases with soft-tissue infections responded fairly well and there was 1 in which the response was doubtful. We had 1 case of cervical lymphadenitis with a very fine response, and 1 case of pyoderma, which also responded. The 3 failures were septicemias and all 3 of these patients died; but I hasten to add that every one of these had a very severe underlying disease with cancer, blood dyscrasia or a major surgical complication, which actually made the recovery less likely. In addition, one of the patients also had enterococcal bacteremia in addition to staphylococcal infection.

I should like to refer for a moment to some bacteriological studies; routinely in our antimicrobial surveys we are getting better than 90 per cent sensitivity of staphylococci to novobiocin at a concentration of approximately 25  $\mu\text{g./ml.}$  of culture media. We have found in a series of 35 different phage type organisms that 26 of them were responsive to less than 0.195  $\mu\text{g./ml.}$  and that many of these organisms showed a bactericidal response to the action of novobiocin.

I thought it would be of interest to show you some of our experiences with the epidemic phage pattern, and in this slide, in which we have 16 different epidemic phage-pattern staphylococci, 42B/52/81 or 80/81, there was one organism that was included just for curiosity because we found it completely sensitive to all common antibiotics, but let me assure you that this is not the routine finding.

The remaining 15 organisms were resistant to penicillin, streptomycin, and tetracycline. Forty-four per cent were sensitive to chloramphenicol, 94 per cent to novobiocin by a pour-plate method. The minimum inhibitory concentration in tube dilution studies was 20  $\mu\text{g./ml.}$  and the minimal bactericidal concentration was 31  $\mu\text{g./ml.}$  In other words,

approximately 50 per cent higher concentrations than the minimal inhibitory concentration were necessary to demonstrate bactericidal action against these particular organisms.

The major limitations to the use of this drug in successful therapy are threefold. First, its toxicity, which is manifested in the form of skin rashes, in perhaps as high as 15 to 20 per cent of the cases reported, leukopenia in an occasional case, pigmentation, and moderate gastrointestinal disturbances. Secondly, resistance of the organism at the concentration that can be achieved in the body is another limiting factor. Thirdly, it appears in reviewing the literature and our own experiences, that poor responses in septicemias or overwhelming respiratory infections may present a patient group in which this drug cannot be expected to exert the maximum effect.

**Dr. Finland (Moderator):** Thank you, Dr. Foltz. I shall now ask Dr. Geraci to give us his summary of the present status of erythromycin, with special reference to the staphylococci.

**Dr. Geraci:** Thank you, Dr. Finland. Erythromycin is an effective antibiotic agent for organisms that are sensitive to its action.

The antibacterial spectrum of erythromycin resembles that of penicillin and covers many gram-positive organisms, particularly staphylococci. It is, however, effective against some gram-negative organisms, such as *Neisseria* and *Hemophilus*. It is of little value against other gram-negative bacteria. Its chief value has been as an effective antistaphylococcal agent for infections caused by staphylococci resistant to other or commonly used antibiotics, such as penicillin, tetracycline, and streptomycin. In clinical therapy, erythromycin is predominantly a bacteriostatic agent, but at times may be bactericidal. With the usual oral dose of 400 or 500 mg. every six hours, only a bacteriostatic effect is achieved in the patient's serum when the antibacterial action is checked against most strains of staphylococci. In vitro, erythromycin apparently may be bactericidal or bacteriostatic, depending upon the sensitivity of the organisms, the concentration of the antibiotic and the maturity of the culture. Erythromycin is active against rapidly multiplying organisms and has little effect on mature cultures. However, for the vast majority of strains of staphylococci, and nonhemolytic streptococci, only a bacteriostatic effect is achieved. In general, the killing effect of erythromycin is not rapid and is quite variable.

Petersdorf and colleagues found that the bactericidal end point for staphylococci that are sensitive to erythromycin was uniformly high. Of the large number of strains of staphylococci that they checked, there were only a few in which the killing end point was the same or close to the bacteriostatic end point.

Erythromycin shows no cross resistance with the other more commonly used antibiotics. However, with other erythromycin-like antibiotics, there is a variable cross resistance. With organisms isolated from clinical material, there is almost complete cross resistance with carbomycin. Partial and variable cross resistance occurs with oleandomycin and spiramycin. With organisms rendered resistant in the laboratory, complete cross resistance was present between all pairs of these agents. The reasons for these differences in the degree of cross resistance between organisms isolated from clinical material and organisms made resistant in vitro are not known.

When erythromycin was first introduced, there were no resistant strains of staphylococci to this agent. At present, this incidence is about 30 per cent at our institution. Apparently, the incidence of erythromycin-resistant organisms parallels the degree to which the antibiotic is used.

Organisms may develop resistance to erythromycin rapidly in vivo, and we have seen resistant organisms emerge as early as 48 to 72 hours after therapy was started. Inasmuch as it has been demonstrated in vitro that this emergence of erythromycin-resistant organisms can be delayed or depressed with combinations of antibiotics, it has been suggested that erythromycin be given in the form of combined therapy.

The in vitro data on combined antibiotic action involving erythromycin may be

summarized as follows: Only rarely does erythromycin enter into synergistic combination with penicillin, tetracycline, or chloramphenicol. However, erythromycin does combine with streptomycin and bacitracin to give bactericidal effects for a number of strains of staphylococci. Behavior of erythromycin in combination with the bactericidal agents is unpredictable and seems to depend to some extent on the sensitivity of the test organisms to the bactericidal agent. However, the only way to know if erythromycin will enter into synergistic combination with another antibiotic agent is to check for the bactericidal effect or activity of the combination in vitro or by means of the serum bactericidal test from serum obtained from a patient being actively treated with the combined agents.

This latter test was used in 16 patients treated with erythromycin in combination with another antibiotic for moderately severe staphylococcal infections caused by penicillin-resistant organisms. The results were in keeping with this generalization. In all instances, the serum of these 16 patients, when tested with erythromycin alone, gave no total bactericidal effect. With four out of nine strains, erythromycin and bacitracin or streptomycin seemed to give synergistic effects, with good bactericidal effect.

Erythromycin is usually administered orally in 400 to 500 mg. doses every six hours. This antibiotic can also be given intravenously, intramuscularly, by aerosol, or instilled into the pleural cavity. Doses as high as 1 Gm. every three hours can be given intravenously for a total daily dose of 8 Gm. with a minimum of side effects. This may enable one to achieve cures that might otherwise not be possible with oral therapy alone. Recently the monoprionyl ester of erythromycin has become available for study. It has been stated that the serum levels of erythromycin are twice as high with this agent with multiple doses as with the enteric-coated erythromycin base. Crossover studies, employing four doses each of the monoprionyl derivative in gelatin capsules and the commercially available enteric-coated erythromycin base in tablets, were carried out in 20 healthy young men. It was found that the average levels obtained two hours after the last dose were 4.5  $\mu\text{g./ml.}$  of serum (range 0.8 to 8.5  $\mu\text{g./ml.}$ ) for the erythromycin base; with the propionyl derivative, the average levels were 5.6  $\mu\text{g./ml.}$  (range 0.5 to 11.5  $\mu\text{g./ml.}$ ). In only about one third of these volunteers were the propionyl erythromycin levels double those of the erythromycin base. The last two doses of the medication were given at midnight and 6 a.m. and were taken in almost all instances on an empty stomach.

Erythromycin is readily absorbed from the upper intestinal tract. Diffusion occurs readily into the various body tissues and cavities and across the mucous membranes of the tracheobronchial tree. However, it does not pass across the uninflamed meninges and into the cerebrospinal fluid. With the usual oral doses of erythromycin, only bacteriostatic amounts for staphylococci are found in the serum, tissues, and body cavities. With the intravenous injection of doses of 0.5 to 1 Gm. rapidly over a 5 to 10 minute period at intervals of three to four hours, extremely high serum levels giving a bactericidal effect may be achieved.

Erythromycin is a relatively nontoxic antibiotic. With multiple doses of 500 mg. every six hours, gastrointestinal irritation and distress occur frequently. With oral doses of 300 to 400 mg. every six hours, distress occurs infrequently. Very little toxicity has been noted with erythromycin. We have seen no skin reactions, drug fever, other allergic manifestations, or other evidence of toxicity. However, urticaria is said to occur rarely.

Erythromycin is of value for mild or moderately severe infections caused by staphylococci sensitive to its action but resistant to the other antibiotics. It is of little or no value when used alone for infections requiring a bactericidal effect, such as endocarditis, osteomyelitis, meningitis, and pyelonephritis. In these infections, erythromycin should not be used alone, unless it can be demonstrated that its use will give a bactericidal effect. When only a bacteriostatic effect may be required for staphylococcal infections, such as in upper respiratory infections, pneumonia, empyema, and abscess formation after surgical drainage, simple bacteremia without bacterial endocarditis, ileocolitis, or in simple urinary tract infections, erythromycin alone in full daily dosage or in combination may be highly effective.

Erythromycin in combination with streptomycin or bacitracin may be used in non-hemolytic streptococcal endocarditis when the patient is allergic to penicillin. Similarly, in patients with pneumococcal pneumonia or sore throat caused by beta-hemolytic streptococci who cannot take penicillin, erythromycin may be used effectively and is said to be only slightly less effective than penicillin.

The treatment of serious staphylococcal infections requires a bactericidal effect. Erythromycin is not the ideal anti-staphylococcal agent. Nevertheless it continues to be a useful antibiotic for staphylococcal infections.

**Dr. Finland** (Moderator): Thank you very much. I want to alert the members of the panel to the fact that I am going to ask each one to interrogate each other on their presentation or to hold them up on some points that have been made if they do not understand or perhaps do not agree.

I am next going to call on Dr. Kirby who will summarize briefly the present status of vancomycin.

**Dr. Kirby:** Earlier in the afternoon, I presented a paper on the current status of vancomycin and I do not propose to repeat the paper. I am sorry you were not all here at that time, but it would be unfair to give it again, so I am just going to make six summary statements about vancomycin.

Our experience and results indicate to us that vancomycin is probably the best agent at present for the treatment of severe staphylococcal infections for the following reasons:

First, it inhibits staphylococci in lower concentrations than any of the other drugs we are talking about.

Second, it not only inhibits them in the test tube but it is more bactericidal than other antistaphylococcal agents.

Third, high effective blood levels can be attained with ease.

Fourth, side effects are minimum. It is inconvenient to administer a drug intravenously, but patients with severe staphylococcal infections are usually in the hospital and need fluids parenterally for nutrition. The incidence of drug fever and rash seems to be quite low but will be known more definitely after a larger number of cases have been treated. We have observed no nephrotoxicity. Deafness has been observed in a few patients with very high blood levels.

Fifth, there is no cross resistance with other antibiotics, and clinical use for two and a half years has not led to the appearance of any antibiotic-resistant strains in our hospitals.

Sixth, vancomycin has been responsible for impressive therapeutic results in a number of cases, particularly the severe ones with staphylococcal endocarditis and other forms of septicemia when the other antibiotics we are discussing have failed.

**Dr. Finland** (Moderator): Thank you very much, Dr. Kirby. I am now going to ask Dr. Quinn to talk about oleandomycin and any other erythromycin-like agent that has not been covered.

**Dr. Quinn:** Thank you, Dr. Finland. Oleandomycin, by virtue of its chemical structure, antimicrobial spectrum, and cross-resistance pattern, has been placed in the erythromycin group of antibiotic agents. Considerable evidence has been accumulated by numerous investigators to indicate that, milligram for milligram, oleandomycin is biologically less active than erythromycin against a number of test organisms including pneumococci, streptococci, and numerous strains of staphylococci. Other studies have shown that the oral administration of oleandomycin as the base, phosphate, or hydrochloride salt resulted in serum concentrations of the agent that were somewhat greater than produced by erythromycin, but not sufficiently high to compensate for the lesser biological activity of oleandomycin. It was further observed that staphylococci, made resistant in the labo-

ratory to either erythromycin or oleandomycin, demonstrated complete cross resistance to the other agent. Because of these considerations, the clinical use of oleandomycin was discouraged.

Another member of the erythromycin group, spiramycin, has been found to have even less antibacterial activity than oleandomycin and is also poorly absorbed following oral ingestion. Clinical trials of this agent have been reported that indicate that it is not so effective as erythromycin.

More recently, a new chemical form of oleandomycin, triacetyloleandomycin, has been reported to be capable of producing much higher serum concentrations than either oleandomycin or erythromycin. Theoretically, an increase in the serum concentration of the agent could either compensate for or exceed its biological inferiority and thus become equivalent or exceed the activity of erythromycin.

Other reports have indicated that while staphylococci display uniform cross resistance to erythromycin and oleandomycin when resistance is induced to one or the other agent *in vitro*, naturally occurring strains of staphylococci frequently demonstrated disassociated resistance patterns. These authors have variously reported that none to 100 per cent of erythromycin-resistant strains of staphylococci were sensitive to oleandomycin. Garrod studied the erythromycin-resistant strains of staphylococci isolated in various hospitals in England with reference to cross resistance to other members of the erythromycin group of antibiotics. He showed that cross-resistance patterns varied from hospital to hospital. Thus, in one hospital almost all staphylococcal strains displayed cross resistance to all members of the erythromycin group, while in another hospital a majority of strains showed disassociated resistance patterns.

It is thus apparent that under certain conditions, triacetyloleandomycin might prove to be a useful antistaphylococcal agent. It would further appear that an indication of the potential clinical value of this agent might be obtained by determining whether triacetyloleandomycin was capable of producing serum antimicrobial activity equivalent to or greater than erythromycin.

Other factors of importance in such an evaluation would be the determination of the actual incidence of erythromycin- or oleandomycin-resistant staphylococci and the cross-resistant patterns of these organisms within the local geographical area.

The first part of the present study carried out by Dr. Colville in our group indicated that within our own geographical area 89 per cent of staphylococci isolated from patients with active staphylococcal infections were susceptible to both erythromycin and oleandomycin. It can be seen from figure 2 (page 405) that the majority of these susceptible strains were eightfold more sensitive to erythromycin than oleandomycin, the average being 9.1. This is in agreement with, and confirms the findings of, other investigators.

Of the 74 strains tested, four were resistant to erythromycin and seven were resistant to oleandomycin. The number of resistant strains was so small that valid conclusions with reference to the incidence of associated and disassociated resistance could not be made. However, it is apparent from these data that complete cross resistance did not occur in either erythromycin- or oleandomycin-resistant organisms, and the implication of this observation would seem to be that in each instance in which either erythromycin or oleandomycin resistance is encountered one cannot empirically select or reject the alternate agent, but that further sensitivity testings would be necessary in order to determine whether cross resistance was present.

The second part of our present study consisted of a comparison of triacetyloleandomycin and erythromycin with reference to serum concentration, serum antimicrobial activity, urine excretion, and urinary antimicrobial activity by means of crossover study in healthy young adults. Figure 3 (page 405) indicates that triacetyloleandomycin produced higher serum antibiotic concentrations throughout the experimental period than did erythromycin.

Serum antimicrobial antibacterial activity studies indicated that despite the previous

observed superior serum concentration of triacetyloleandomycin the antibiotic activity of the serum after ingestion of this agent was still slightly less than that observed after a similar dose of erythromycin. The figures to the right in figure 5 (page 406) indicate that the area under the curves, measured as dilution hours, are also greater for erythromycin. These observations were consistent following single doses of 500 or 1000 mg. of the agent.

In another crossover study with 500 mg. multiple doses similar findings were obtained. Variations of the test organisms *Sarcina lutea* in the first study and *Staphylococcus aureus* in this study indicate that the comparative results of serum antibacterial activity is inferior for triacetyloleandomycin for both test organisms.

These observations with reference to the comparative serum antimicrobial activity are in essential agreement with those reported by Reisch and co-workers and by Kunin and associates.

Results of our preliminary studies employing erythromycin propionate are similar to those reported this morning by Dr. Kirby and by Dr. Kunin, namely, that this agent is capable of producing much higher blood levels and serum antimicrobial activity than erythromycin stearate. By virtue of this apparent increase in absorption, serum antimicrobial activity following erythromycin propionate was much greater than that observed following similar doses of triacetyloleandomycin.

Urinary excretion studies indicated that about 18 per cent of the ingested dose of triacetyloleandomycin and about 1 per cent of the dose of erythromycin was excreted in 24 hours. The antimicrobial activity of the urine was approximately twice as great for triacetyloleandomycin.

Since the true test of the clinical worth of an antimicrobial agent depends upon its effectiveness in the actual treatment of disease, and this must be determined by carefully controlled studies, it is not possible from this type of study to draw conclusions as to the real value of triacetyloleandomycin, as an antistaphylococcal agent. However, this type of study does permit certain limited conclusions: (1) The use of either triacetyloleandomycin or erythromycin for the treatment of staphylococcal infections would seem to depend largely on the antibiotic susceptibility characteristics of the *Staphylococcus* population in the particular geographic area.

(2) Resistance of staphylococci to either erythromycin or oleandomycin does not imply either resistance or susceptibility to the other agent, and selection of the proper agent should therefore depend on sensitivity studies.

(3) Despite its ability to produce higher serum concentrations, triacetyloleandomycin induces serum antibacterial activity, which is somewhat less than that produced by an equal dose of erythromycin. On this basis, it would appear that in staphylococcal infections due to organisms that are sensitive to both agents erythromycin would be the agent of choice.

**Dr. Finland** (Moderator): Thank you very much, Dr. Quinn. Now I shall call on Dr. Romansky who will review for us the status of ristocetin as an antistaphylococcal agent or as an antibacterial agent.

**Dr. Romansky:** This morning there were five papers in which I think very successful results were reviewed in the very overwhelming staphylococcal infections. In these cases ristocetin was used intravenously as it has been previously. The general range of dose schedule has been 25 to 50 mg./Kg., usually in two doses a day, given in solutions varying from 20 to 100 ml. over a period of five minutes to 20 to 25 minutes. I think those of you who saw some of the patients who were treated, as mentioned this morning, were amazed that these patients recovered. Dr. Kirby's cases are probably very similar. As a matter of fact, most of us who have been treating the staphylococcal infections in patients have been dealing with the almost terminal cases, which makes the results even more remarkable.

Two years ago we first reported on the effectiveness of ristocetin in pneumococcal pneumonia as well as in other gram-positive infections, and just so it is not forgotten, ristocetin was also effective in severe gonococcal arthritis. Laboratory results indicate that most of the gram-positive organisms are inhibited by small amounts of ristocetin, usually less than 5  $\mu$ g., and that in general the bactericidal level is rather close to the bacteriostatic level. These include serum bactericidal levels.

At the last Antibiotics Symposium we reported on the successful short-term therapy of enterococcal and staphylococcal endocarditis with ristocetin in 7 patients, and since that report, I think it is worthwhile to mention that we have completed treatment in 14 patients. Of these 14 patients with endocarditis, there are 9 with enterococcal, 2 with alpha-hemolytic streptococcal, 2 with negative blood cultures, but a classical picture, and 1 with hemolytic *Staph. aureus* endocarditis. Treatment was successful in 13, and equivocal in 1 patient with alpha-hemolytic streptococcal endocarditis. We might say, these results are rather striking in their success, particularly with the enterococcus, because we now, in my opinion, have a means of treating enterococcal endocarditis over a two week period with approximately 2 Gm. a day or 28 injections. In view of the results in staphylococcal endocarditis, we felt that subsequent studies were indicated in other types of staphylococcal infections.

As you probably know, at the last Symposium, Taylor, Schumacher, and Calvy reported on the successful results in 4 patients with severe staphylococcal infections treated with ristocetin. Since that Symposium, in addition to the cases already mentioned this morning, we have had occasion to treat a total of 10 patients with very severe infection, which included 4 with staphylococcal pneumonia, 3 with multiple abscesses of whom 1 had sepsis, 1 with a brain abscess and meningitis, and of the remaining 2, 1 had a postoperative wound infection with an extremely critical course, and the other a puerperal endometritis with septicemia. Of the 10 patients, 8 had other antibiotics that had not been effective prior to administration of ristocetin; 8 of the 10 patients received 25 to 50 mg./Kg. of ristocetin on the schedule I mentioned a short time ago; of the remaining 2, the 1 with the overwhelming puerperal endometritis and septicemia received 9 Gm./day and the patient with brain abscess and meningitis received 6 Gm./day. Total doses ranged between 7 and 72 Gm. over a 3½ to a 15 day period in 9 patients. The patient with the severe postoperative wound infection following cholecystectomy had one course of 16½ days of ristocetin, then an antibiotic-free interval of 10 days followed by a course of 29 days of ristocetin. This patient had 28.5 Gm. in the first period, and 36 Gm. in the 29 day period, for a total of 64.5 Gm.

A good response was obtained in 8 of the patients. Some explanation is needed for the other 2. Blood cultures of the patient with puerperal endometritis and septicemia became negative, subsequent to the administration of ristocetin. This case indicates that the surgeon is an important part of our treatment of staphylococcal infections. In this particular instance this patient had many antibiotics prior to the administration of ristocetin. Also the blood cultures were positive up until the time ristocetin was administered. The blood cultures then became negative. The patient was so toxic that it was necessary to use steroids, and the temperature subsided; however, as soon as the steroids were discontinued, the temperature again rose and at this time a hysterectomy was done and a necrotic uterus removed. I would only like to point out that the presence of foci is a common problem in the very serious situation of staphylococcal infections, and I think this patient illustrates here that we might have gone on indefinitely using various agents singly or in combination, but until that uterus was removed we would have continued to have a continuous febrile response.

The patient who had the two long courses of ristocetin postcholecystectomy had a remission but recurrence of abscesses until ristocetin was again initiated. With various incisions and drainages and continuation of ristocetin a good response occurred.

We had a patient recently who also illustrates a problem that all of us have seen, that is, severe septicemia in which the initial response to an antibiotic was a negative blood cul-

ture. With reduction of the dose from 3 to 2 Gm./day, the blood cultures in this patient again became positive and subsequent increase in dose was of no avail. The in vitro tests revealed the organism to be responsive to ristocetin and subsequent bactericidal tests also revealed this to be so. Just why a result could not be obtained despite the in vitro test, I cannot explain at the present time. This circumstance is not unknown, since I am sure we are all familiar with the failure of therapy in patients with penicillin-sensitive staphylococci. For example, the first patient that we reported with staphylococcal endocarditis responsive to ristocetin did have an organism responsive to penicillin but did not respond to adequate penicillin therapy.

In this group of 10 patients, the usual blood counts and other studies were carried out and no adverse effect was noted on the hemological system, and this despite the fact that one patient had 6 Gm./day for 12 days and another 9 Gm. for 7½ days.

What we have seen in the way of side effects is what has already been reported by us and others. We had 2 patients in the endocarditis group who had a skin rash from ristocetin. We have seen transient neutropenias. We have had 1 patient with a thrombocytopenia that responded upon cessation of the dose of ristocetin. We have 2 patients who have had rather severe nausea and emesis upon the administration of ristocetin and when the dose was decreased this also disappeared.

On the basis of the studies that have been reported today and our own studies, it would appear that ristocetin is a most effective agent in severe staphylococcal infections. If given within the range of approximately 25 to 50 mg./Kg., side effects generally do not occur, and even with larger doses side effects need not present themselves. It is important to bear in mind that in the presence of renal insufficiency the dose should be decreased, since concentrations in the blood will become excessive.

**Dr. Finland (Moderator):** Thank you, Dr. Romansky. The final panelist, Dr. Yow, is going to summarize his view of the status of kanamycin.

**Dr. Yow:** Kanamycin has now been tried clinically in this country for approximately 10 months. At first it was tried rather cautiously in highly selected cases, and more recently in a large number of patients with a greater variety of infections. I would like to summarize rapidly the pharmacological and clinical information concerning this antibiotic based on reports appearing in the medical literature and observations made on the treatment of approximately 200 patients in Houston.

Kanamycin is rapidly absorbed and excreted following intramuscular administration at a rate similar to that of penicillin. It is excreted by glomerular filtration. Its absorption from the gastrointestinal tract is extremely poor producing blood levels in the range of 2 to 7 µg. following oral doses as great as 4 Gm. It is quite soluble in water and is extremely stable, probably the most stable antibiotic in general use today in that it can be autoclaved, and it can be boiled for 30 minutes.

The organisms that are usually sensitive to kanamycin include the *Staphylococcus*, the coliform organisms, the *Aerobacter-Klebsiella* group, anthrax bacillus, gonococcus, *Salmonella*, and *Shigella*. Most strains of *Proteus* are sensitive to kanamycin. A few strains of *Pseudomonas* are sensitive, but most strains are resistant. The organisms that are usually resistant include the pneumococcus, most of the streptococci, the fungi, and most of the anaerobic organisms, including the bacteroides, the clostridia, and anaerobic streptococci. We have tested one strain of *Brucella abortus*, which was found to be sensitive in vitro but the patient did not respond to kanamycin therapy.

There is a complete crossover in sensitivity between kanamycin and neomycin. All of the strains of staphylococci have been sensitive to both kanamycin and neomycin but strains of the coli-aerogenes group resistant to neomycin are also resistant to kanamycin and strains sensitive to neomycin are sensitive to kanamycin. There is the same crossover in the *Proteus* and the *Pseudomonas* groups.

There is a partial, or one way, crossover with streptomycin, but strains of bacteria resistant to streptomycin are often sensitive to kanamycin.

Already there has been some disagreement today among members of the panel concerning bactericidal and bacteriostatic concentrations with various antibiotics. In our own experience, all of the strains of staphylococci tested have been inhibited by 6.2  $\mu$ g. of kanamycin per ml. or less, using heart infusion broth. The difference between the "static" and "cidal" concentration has been no greater than 1 dilution.

Quite a wide variety of infections have now been treated with kanamycin. It has been effective in the treatment of most infections due to the *Staphylococcus*, including many serious infections. However, kanamycin is often ineffective in sterilizing localized infections, such as empyemas, large abscesses, and meningitis. Additional local therapy, surgical drainage, or instillation of the drug into the cavity may be necessary in these conditions. It is difficult to evaluate the effect of the therapy of osteomyelitis because of its variable natural history but preliminary reports of the effect of kanamycin in acute osteomyelitis have been quite encouraging. Most of the infections due to *E. coli* such as pyelonephritis have responded dramatically. Most infections due to the *Aerobacter-Klebsiella* group of organisms have responded quite well, as well as many infections due to *Proteus*. This latter organism is of interest, of course, because of the fact that *Proteus* strains are notoriously resistant to most of the other antibiotics. A fair number of miscellaneous infections have been treated, including anthrax, gonorrhea, salmonellosis, shigellosis, with favorable results. At present we have a patient under therapy with acute endocarditis due to *Salmonella panama*, who apparently is responding quite well to kanamycin. We have another patient who had recurrent salmonella enteritis with persistently positive stool cultures, who failed to respond to many of the antibiotics who has now been clear for more than six months.

There are other reports of favorable effects in *Salmonella* infections, but the reports of the use of kanamycin in typhoid fever have been somewhat variable. Some patients have improved and some have not.

Most pneumococcal and streptococcal infections have not responded favorably to kanamycin therapy. Many cases of typhoid fever and brucellosis have not responded well. Most infections due to *Pseudomonas* have not been altered by kanamycin therapy, though an occasional patient has responded dramatically. Many *Proteus* infections have been cured by the administration of kanamycin but infections due to yeast and anaerobic organisms have not responded.

When the drug is given orally for sterilization of the bowel, anaerobic organisms and yeast usually persist.

There is some variation in dosage in kanamycin utilized in different centers at the present time. We have used 3 Gm. daily for short periods in severe illnesses, 2 Gm. in moderately severe cases, and 1 Gm. in mild cases. In children we have used doses as large as 100 mg./Kg. for short periods but usually 50 mg./Kg. in severe cases, 25 and then 12.5 mg./Kg. in milder cases. Doses of from 4 to 8 Gm. have been given daily for sterilization of the bowel but of course this has no influence on systemic infections under most circumstances. Kanamycin may be used locally in 2.5 to 5 mg./ml., or even greater concentrations under certain circumstances.

There is little doubt that there is a definite toxicity associated with kanamycin therapy, and this was reviewed very well earlier in the meeting by Dr. Finegold.

The real significance of renal toxicity is difficult to evaluate at the present time. There is definite evidence that kanamycin has some irritating effect on the kidney. How serious this is is unknown, but apparently it is not a frequent problem clinically. We have recently treated a patient with a creatinine of 18 with 1.5 Gm. of kanamycin a day for about two weeks without a significant increase in creatinine or without any decrease in urinary output. This I would say was a rather severe test. Time will tell, of course, how serious this potential complication is.

Auditory loss is definite. It seems to be related closely to the amount of drug given and duration of therapy, but also the rate of excretion. Preliminary studies from several places have indicated that in patients with renal damage the drug may not be excreted

as rapidly and the appearance of auditory loss beginning in high frequencies and working down to mid-range and finally lower frequencies is more common. In our own experience this problem has occurred most frequently in diabetics and particularly in diabetics with Kimmelstiel-Wilson disease. This is as you would expect, since this is a disease that affects primarily the glomerulus. The drug is excreted by glomerular filtration. Consequently interference with excretion would be likely to be greater in this disease. We have seen 2 patients who have developed total deafness, and 3 others who in all probability had hearing loss due to kanamycin, but who also received other potentially ototoxic drugs. Undoubtedly, many other patients have had loss in high frequencies but because of the problem of detecting slight changes in hearing in patients that are critically ill, we are not too sure about this point. Occasionally eosinophilia has been noted; this apparently is of no consequence.

In summary, then, kanamycin appears to be one of the most important contributions to the antibiotic group of antibacterial agents for use in serious infections developed in recent years. It has the advantage of being active against virtually all strains of staphylococci resistant to other antibiotics, and against many antibiotic-resistant strains of the gram-negative bacilli. It is rapidly absorbed following intramuscular injection, but not absorbed from the gastrointestinal tract. Its greatest limitation appears to be that of the auditory toxicity that is so frequently associated with administration of large amounts of the drug. There is suggestive evidence that the drug may be nephrotoxic, but this has not been a frequent problem clinically thus far. Because of these potential toxic properties, the drug should be used with care and for specific indications.

**Dr. Finland (Moderator):** Thank you, Dr. Yow. I think we have had the opinions of each of these specialists about an individual drug, and before proceeding to interrogate them as to just how they really work in the clinic, I am going to give the individual members of the panel an opportunity to ask any questions or try to get any elucidations of points that might not have been clear to them in the presentations that have already been given.

Dr. Romansky.

**Dr. Romansky:** I would like to ask Dr. Geraci whether he feels that the unquestionable pharmacologic advance noted with the introduction of the propionyl derivative of erythromycin enhances its use in the management of the moderately or the more resistant staphylococcal infections?

**Dr. Geraci:** Well, I think that it would, because it has already demonstrated that the antibacterial action is increased with the propionyl derivative. In other words, the action would be greater with the propionyl derivative than with the erythromycin base, so I think that it probably would help.

**Dr. Romansky:** One point I meant to mention. I did not hear Dr. Foltz say that with novobiocin one occasionally gets elevation of the serum bilirubin, but this is not related to any hepatic dysfunction.

**Dr. Foltz:** We have had 1 patient who had a novobiocin rash, and he was continued on his novobiocin for 11 days after this occurred. At this time he had a markedly elevated bilirubin, and a biopsy showed what was compatible with an intrahepatic obstructive jaundice such as you see with chlorpromazine.

**Dr. Finland (Moderator):** Dr. Yow, did you want to make remarks on that?

**Dr. Yow:** I think it is quite right to draw attention to the potential hepatic toxicity here, but I think this should be only a minor deterrent in the use of novobiocin; actually

there is a great deal of question as to the significance of this bilirubin finding, I think, as far as hepatic toxicity is concerned.

**Dr. Finland** (Moderator): Dr. Foltz.

**Dr. Foltz:** I would like to ask Dr. Quinn whether he would employ triacetyloleandomycin, clinically, when a therapeutic agent is required in moderately severe staphylococcal infections, especially if erythromycin resistance is noted in the local scene.

**Dr. Quinn:** I think it is preferable, as we pointed out, that in each instance the sensitivity of the organism should be determined because one cannot predict the presence or absence of cross resistance. Certainly if in your local area the evidence was in favor of a lack of cross resistance, this would give added reason to consider use of this agent.

**Dr. Finland** (Moderator): Are you satisfied, Dr. Foltz?

**Dr. Foltz:** Yes, I think I would agree on that because during the past winter we had seen an amazing amount of erythromycin resistance. We have employed triacetyloleandomycin in a very limited pediatric study and found rather encouraging responses here, and I would modestly vote for the employment of it under these circumstances.

**Dr. Finland** (Moderator): Now, we are really getting to the heart of the problem. Each one presented his views about one particular antibiotic. Each one of them is a physician who is, or might be considered, an authority in his own right and by virtue of his experience. So I am going to pose a question that I am sure all of you would like to hear discussed by each of these distinguished panelists. The question is, how do you manage a staphylococcal infection in your hospital. I shall start with Dr. Kirby.

**Dr. Kirby:** Let me first say this, though, that I think the trouble with comparing a list of drugs and the trouble with evaluating staphylococcal infections in general is that they are either too easy or too difficult. The too easy ones are the superficial infections where drainage is perhaps the most important thing, and it is certainly difficult to compare them in these instances. The too hard ones are those in which the patient has abscesses deep in his tissues, in the spleen, and so on, and is going to die no matter what he gets. This complicates the clinical evaluation, but I think on the basis of the kind of evidence we have given you some comparisons have been made and can be made; and I would just like to write down in the order in which I would select these, and make a statement or two about them.

This would be my list: I would say that for a *severe* staphylococcal infection in a hospital, that might be life-threatening, or that might be going to become severe and form abscesses and become untreatable, to me at the present time, the first antibiotics to use would be vancomycin or ristocetin, and we are assuming that these are severe hospital infections in which the organisms are resistant to penicillin, tetracyclines, and streptomycin, as most of them are.

These would be the main choices then. The next drugs in order would be erythromycin, novobiocin, kanamycin, oleandomycin, and I have included spiramycin, since it was discussed today, but, where possible, the latter ones would be avoided for the most severe infections. I would say, then, to make an analogy that although I think vancomycin and ristocetin are quite close, I would consider vancomycin, the Cadillac; ristocetin, the Oldsmobile; erythromycin would be the Pontiac; novobiocin, the Chevrolet; and kanamycin, the Ford.

**Dr. Finland** (Moderator): Well, some of us drive Fords. Dr. Quinn will now give us his

idea as to how he would manage a case. We are talking now really only about severe staphylococcal infections, with these or any other antibiotics.

**Dr. Quinn:** I would like, first of all, to make the point that although the penicillin-susceptible staphylococcal case is now less common, we should always keep it in mind. If the organism is sensitive to penicillin, I certainly think it would be the antibiotic of first choice. I would rank the seven antibiotics under consideration as follows: ristocetin and vancomycin in the first group, kanamycin and novobiocin in the second, and erythromycin, oleandomycin, and spiramycin, in that order, in the third group.

**Dr. Finland (Moderator):** Dr. Romansky.

**Dr. Romansky:** I do not differ too much from the previous two, since we are talking about severe infections, and I would put ristocetin first, primarily because of my own clinical experience. I do think that ristocetin, vancomycin, and kanamycin should be grouped, and I would group the others, erythromycin, chloramphenicol, novobiocin, triacetyloleandomycin in that order.

**Dr. Finland (Moderator):** Dr. Yow.

**Dr. Yow:** I would like to differ a little with Dr. Kirby. In the first place, I happen to feel rather strongly that in certain patients with severe staphylococcal infections the agents that are most actively bactericidal against the largest numbers of organisms are the ones that are most likely to eradicate the infection. And I would agree that vancomycin probably is the most active antistaphylococcal drug that I have used. I do not think it is a great deal more active than kanamycin or neomycin, in my experience. Vancomycin has certain other difficulties in that it has to be given by intravenous drip, which creates problems in certain groups of patients, particularly newborn infants and young children; it creates problems in patients who have had extensive surgical procedures, so that intravenous administration is not easily carried out. I think also that bacitracin ought to be included not too far behind this first group of drugs. It apparently is active against all, or virtually all staphylococci, and then, from my experience, I believe that ristocetin and novobiocin both should be put in a group of intermediary drugs that are not highly active as bactericidal agents. They are more likely to leave organisms that persist during our therapy than would others. There is also chloramphenicol, which should be considered as an active agent. Most of our strains are susceptible to chloramphenicol, but it is primarily bacteriostatic. My big difference with the rest of the group about ristocetin is based primarily on personal experiences and a few cases that have been treated with doses as large as 4 Gm./day of ristocetin by intravenous drip who have had persistently positive blood cultures for staphylococci and whose cultures have been sterilized by the use of both kanamycin and vancomycin.

**Dr. Finland (Moderator):** Thank you, Dr. Yow. Dr. Foltz.

**Dr. Foltz:** Well, I would also like to try to get some clinical evaluation into this picture of selection of drug. I would go along with Dr. Kirby and Dr. Yow that vancomycin certainly has been, and is, in our experience the most effective agent in the very severe cases of staphylococcal infection. We have had very limited experience with ristocetin and kanamycin, but I think ristocetin certainly belongs in the same group with vancomycin. We have also used bacitracin in our severe cases with a fair degree of success. I would put novobiocin very close to the top, and I think it should be used in conjunction with some of these other agents, because, as we found, it has been bactericidal against many of our epidemic strains and against the organisms that we are isolating from our serious infections. We also use chloramphenicol for the moderately severe cases and like to use

it in conjunction with novobiocin in such cases. Certainly, our simple cases that do not show immediate response to surgical handling or where there has been a cellulitis or lymphangitis complicating the local picture, we find that penicillin or chloramphenicol in combination have been extremely useful in the management of these infections.

Erythromycin has been ineffective in our hospital for the past six months to a year because of the marked resistance shown by the organisms that we have to deal with, and I do not know yet exactly what the position is of oleandomycin but I think it might very well be ranked as one of the drugs to be used in moderately severe infections.

**Dr. Finland (Moderator):** Dr. Geraci.

**Dr. Geraci:** I think I would agree with most of what has been said. I would like to confirm Dr. Kirby's findings with vancomycin. As of this time, we have treated 44 patients with vancomycin, 14 reported previously in the last year or so. Six of these have been cases of *Staphylococcus* endocarditis, and when we think of serious staphylococcal infections, we think of infections that require bactericidal effect, such as meningitis or endocarditis. In these infections vancomycin is, I think, the agent of choice. In our 6 cases of staphylococcal endocarditis, I think we can say there were cures in 5 of the 6, which represents a good result. Four of these are still living; 1 of them died two or three weeks after therapy was concluded, but at postmortem examination, all cultures of the blood and heart valves were negative. One patient died during therapy.

**Dr. Finland (Moderator):** Thank you, Dr. Geraci. I think there's probably a larger percentage of concurrence than of differences expressed among the panelists. However we are not through yet, and I hope that some battle will develop here in one area or another. The question I would like to pose to the panelists next is: How do they use the laboratory in the management of their cases of staphylococcal infections? And I am going to start with Dr. Yow.

**Dr. Yow:** I suppose this particular point I wish to make is not what you are really referring to, Dr. Finland, but I think it is worth mentioning. That is, today we are seeing new antibiotics being introduced that are potential antistaphylococcal agents, and those of us who are interested in the field are obligated to continue to study them and try to make comparative studies in the laboratory that will aid in guiding our clinical therapy. I think that goes without saying. In addition to this, there are considerable strain variations in susceptibility of staphylococci to certain of the antibiotics, so that careful determination of a specific strain's susceptibility is important. There are also some differences in the bactericidal effect and the bacteriostatic effect depending on strain differences, which may be determined in the laboratory during therapy. In addition, the technique that Dr. Geraci mentioned this morning and Dr. Finland has mentioned on previous occasions, of studying the patient's serum during therapy, to determine the bactericidal effect of the serum against the organism producing the disease in the individual patient will often give one information about the likelihood of the patient's relapsing or the likelihood of the patient's being completely cured rather than waiting for two or three weeks to complete a course of therapy. I think those are the main points, other than routine cultures and the routine studies that we would use the laboratory for.

**Dr. Finland (Moderator):** Dr. Foltz, do you want to give us more details as to just how you go about it? What do you require of a laboratory in the management of a case from its beginning to end?

**Dr. Foltz:** I think the first thing that we want to know from the laboratory is the identity of the infecting organism, and whether we are dealing with, first of all, a pure culture or

a mixed culture. I think this has much to do with our decisions as far as therapy is concerned. Second, I would like the laboratory to back me up in checking on the clinical improvement by noting whether there have been any changes in the flora that we can collect from the various areas, or fluids that are related to the infection. Third, perhaps last, and certainly not least, I would like to know about the sensitivity phenomena, but I would like to know this mainly to help me in selecting, as soon as I have made the clinical diagnosis of a staphylococcal infection, what the best choice of drugs should be. I think that this can be obtained better by using large series over a period of weeks or months in the terms of sensitivity results, rather than taking the result to the individual organism. If a doctor is going to sit back and wait six or eight hours for a very short term sensitivity test, or wait until 24 hours have elapsed to make the choice of a therapeutic agent, he is going to miss a golden opportunity in the management of these staphylococcal infections. Therefore, I think the knowledge of what is the prevalent sensitivities in the given area or hospital is far more important than the individual drug sensitivity for the organism that you are dealing with, in this particular infection, at least for initiating therapy. Certainly, he would like to know, within 48 hours at least, what the particular organism responds to, but by that time you should have some clinical impression of whether your therapeutic choice has been wise or not. I think that this is the role that the laboratory should play in the over-all management of staphylococcal infections.

**Dr. Finland (Moderator):** Dr. Kirby.

**Dr. Kirby:** I would just like to add one point. We have for several years used a single disc antibiotic sensitivity testing method with measurement of zone diameters. We have recently completed, or are completing, a review of thousands of strains so tested, and there is more than 90 per cent accuracy in selecting the resistant versus the sensitive organism. These data will not be presented at this meeting, but I think one of the most important things at the present time is the dissemination of more reliable information concerning proper performance and interpretation of disc sensitivity tests.

**Dr. Finland (Moderator):** Dr. Quinn.

**Dr. Quinn:** I would like to add just a rather simple thing, namely, that the laboratory results will be accurate only if the specimens are properly collected and delivered. I think it is the clinician's responsibility to see that this is carried out.

**Dr. Finland (Moderator):** Dr. Romansky.

**Dr. Romansky:** This, too, is just a simple thing, but always in my opinion there ought to be more than one blood culture and the media to which it is dispersed should be at least duplicated or triplicated, that is, there ought to be broth, anaerobic media, and pour plates. One other point that we have made for years is that so many patients prior to admission have received antibiotics, that frequently it is difficult to get the organism to grow because of the mere presence of the antibiotic. What we do in one of our media is to dilute the broth five times in the presence of the same amount of blood, and occasionally we will get a positive culture by virtue of just diluting out the antibiotic.

**Dr. Finland (Moderator):** Thank you, Dr. Romansky. It is obvious that everyone here uses the laboratory and more or less depends upon it, and I think the emphasis is on the intelligent use of the laboratory, both for the diagnosis of the disease, for the establishment of the most effective and useful antibiotic, and in the conduct of the case as it progresses.

Now, we have heard in the previous discussion a great deal said about vancomycin, and in a Panel Discussion tomorrow, there will be a good deal of discussion about

staphylococcal infections. In order not to overlap too much, we are not really discussing the entire problem of staphylococcal infections, and I would like to have that made very clear. These are only discussions concerning the use of antibiotics in certain types of infections. The impression might have been gained that these drugs, if you just start from your choice at the top and many of the panelists have chosen vancomycin, are the answers to the problem of staphylococcal infection that is troubling the community and particularly the hospital staphylococcal infections. I would like to start with Dr. Kirby and ask him merely to put this in its context with respect to the problem of staphylococcal infections in hospitals.

**Dr. Kirby:** Well, it is such a big subject that I shall make only a few points. One is that at the present time in hospitals there are debilitated patients who earlier would have died of diseases and who are now living much longer. They are very susceptible to these infections. The other point is that much of it comes down to housekeeping in the hospital: housekeeping and proper protection of the environment and isolation of patients. In city hospitals, such as the one in which I work it is absolutely impossible at present to isolate all of the patients with open active staphylococcal infections.

**Dr. Finland (Moderator):** Dr. Geraci, will you relate your experience with vancomycin or any other antibiotic in relation to the general problem of staphylococcal infections and our hospital's problems?

**Dr. Geraci:** Well, I think what Dr. Kirby has said is certainly so. Most of the hospital staphylococci are resistant to penicillin and to tetracycline and to streptomycin, and other than utilizing the isolation of the patient and aseptic technique, I think that use of one of the bactericidal agents for treatment is what is necessary.

**Dr. Finland (Moderator):** Well, the point is that this is a drug that is useful after somebody has already acquired a serious infection in the hospital, but it does not really solve the problem that is involved in our hospitals today. Does that express your opinion? Dr. Foltz, do you want to add anything?

**Dr. Foltz:** I would like to put in a word for avoidance of the prophylactic use of antibiotics. I think this has been something that has been entirely overworked. It is my personal belief that more antibiotics are used in hospitals at the present time as hypnotics and sedatives for the attending physician than actually for the prophylaxis of infections in patients.

**Dr. Finland (Moderator):** Thank you. Dr. Yow.

**Dr. Yow:** I would just like to add that from the point of view of therapy most staphylococcal infections can be controlled, that is, mortality can be prevented. Morbidity, of course, is a difficult problem, and unless the infections themselves can be prevented, then this will not be solved.

I would like to relate just briefly an incident in Houston. Most of you know about the epidemic there, and the high mortality rate among newborn infants. This was largely controlled, from the point of view of mortality, though the incidence of infection has remained much higher than we would like. Then, following the first of July, we got a new group of interns and residents, all of whom were graduates of good medical schools, but many of them had some of their education concerning treatment of infections from advertising literature rather than from sound teaching of principles, I am afraid. The mortality rate again rose: for they were using the latest antibiotics and often the most expensive ones, but not necessarily the most effective ones.

**Dr. Finland (Moderator):** Dr. Romansky.

**Dr. Romansky:** I would just like to re-emphasize again the need for the surgeon's being aware of our staphylococcal problems, because the antibiotics are not the complete answer to this particular situation, as was demonstrated in one of the cases I presented. There may be frequent occasions when there will be a mad chase from one antibiotic to another, but there may be a quart of pus somewhere in the fascial plane or in a muscle that urgently needs drainage, and I think this should be emphasized just as much as the definitive use of a specific antibiotic.

**Dr. Quinn:** I think it is really important to place emphasis on the education of the staff; this is where solution of this problem begins, and we must take this very seriously.

**Dr. Finland (Moderator):** I did not mean to enter into the province of the next panel, so we shall go back here to our problem. One of the problems that present themselves was raised by Dr. Quinn; we have a list of antibiotics and penicillin was not on that list. Dr. Kirby introduced bacitracin into that list, and very properly, and that was discussed by others. Now, I would like to pose a specific question to the members of the panel, because there are differences of opinion. With respect to organisms that are moderately resistant or resistant to penicillin, I would like to ask each member to tell us how he feels about the use of large doses of penicillin in such infections in which, in the patient, all the organisms are penicillinase producers. Dr. Foltz.

**Dr. Foltz:** We have tried massive doses of penicillin, and I am unable to give you a definite statement about this, but it is my impression that we have not accomplished very often cure of the patient by high doses of penicillin. We have generally had to go to a more effective drug.

**Dr. Finland (Moderator):** Dr. Geraci.

**Dr. Geraci:** I would agree with this. In the series of 28 cases of staphylococcal endocarditis that we have reported, the only cures that we achieved with penicillin or penicillin-streptomycin together with probenecid were in those cases in which the organism was penicillin sensitive, and there were only 5 of those.

**Dr. Finland (Moderator):** Dr. Kirby agrees. Dr. Quinn.

**Dr. Quinn:** I agree only in the clear-cut penicillin-sensitive group.

**Dr. Finland (Moderator):** Dr. Yow.

**Dr. Yow:** I would certainly agree with the rest of the panel. I think there may be an occasional case in which the organisms require something like 2 units, or maybe 3 units; if the patient is treated early, with extremely large doses, he may be cured.

**Dr. Finland (Moderator):** Now, is that a resistant strain or is it a sensitive strain?

**Dr. Yow:** Well, that depends on who is talking about it—it is a borderline strain.

**Dr. Finland (Moderator):** Thank you, Dr. Yow. I think there is a general agreement here, at least among the members of the panel, that when they are dealing with a penicillinase producer they would not recommend the use of large doses of penicillin as an additional agent as an adjunct to the treatment of severe staphylococcal infections due to such organisms.

Now, I would like to ask the panel, and this may come up again, whether any of them have any place for the sulfanilamide drugs in the management of moderate or severe staphylococcal infections. Dr. Yow.

**Dr. Yow:** I do not believe it has any place.

**Dr. Finland** (Moderator): There is universal agreement this time, too, I guess. Perhaps we can raise some problems now, where there is not so much agreement. Some of you know that I am supposed to be the one who says that you should never use antibiotic combinations. I do not believe I ever said that, and I do not imply that, and I rarely treat a patient with severe staphylococcal infections with a single drug. Now, I would like to ask the members of the panel, individually, to tell us how they feel about the use of *single* drugs in the treatment of severe staphylococcal infection, and what limitations they place on the use of single drugs. We shall start with Dr. Geraci.

**Dr. Geraci:** Well, if the organism is penicillin sensitive, then I think that I would use penicillin alone and perhaps try to elevate the serum levels with probenecid. I think if the organism is penicillin resistant, then I would go to vancomycin, as has been mentioned, and I would use vancomycin alone. I think that in the few reports that have appeared in the literature and in the few tests that we have done on patients being actively treated with vancomycin, there has not been too much demonstrated in the way of enhanced killing effect with combinations with vancomycin. I think the same perhaps can be said with ristocetin. I would probably use ristocetin alone. In connection with the other agents, novobiocin and erythromycin and oleandomycin, I would probably use those in combination preferably with bacitracin or streptomycin because in the limited studies available, it seems that these agents enter into bactericidal combination with erythromycin in the treatment of active infection more often than with the other killing agents.

**Dr. Finland** (Moderator): I presume that you meant provided that the organism is sensitive to these other agents?

**Dr. Geraci:** Yes.

**Dr. Foltz:** Well, as far as we are concerned, we have not noted any staphylococci that have been resistant to vancomycin, as Dr. Kirby said earlier in the afternoon. All of our organisms have been inhibited by 5  $\mu$ g., and usually the bactericidal end point is the same, or only one dilution, or one concentration greater.

**Dr. Finland** (Moderator): Well, I was referring to the use of streptomycin and bacitracin as adjuvants or additional second agents when you use agents like vancomycin on the one hand and when you use novobiocin or erythromycin, on the other.

**Dr. Geraci:** Yes, except perhaps in the case of bacitracin. Even though the organism might be very resistant to bacitracin, in some instances the combination of bacitracin and erythromycin has been bactericidal.

**Dr. Finland** (Moderator): Dr. Foltz.

**Dr. Foltz:** I would agree on the use of two antibiotics when the organism is susceptible to the action of both. I think that we are certainly justified in using antibiotics singly during the period of study, but, since the staphylococci have all shown the ability to acquire or to emerge as resistant to each of these agents singly when they are used, preponderantly in one locale, I think that it is wise now to have some sort of systematic approach to combined therapy as a hope of delaying the emergence of these resistant organisms.

There are few clinical studies, many test tube studies, but I think final proof would be in carrying the studies out at the clinical level rather than making the decision primarily on our in vitro studies.

**Dr. Finland** (Moderator): Would you include vancomycin and ristocetin among the agents that you would use in combination, or would you agree with Dr. Geraci that those can be used alone?

**Dr. Foltz:** We have used each of those agents alone, but in recent months, in our severe septicemia cases we have not hesitated to add novobiocin or chloramphenicol when either of those agents have proved to have an effective action on the *Staphylococcus* in question.

**Dr. Finland** (Moderator): Dr. Quinn.

**Dr. Quinn:** I would agree. However in the investigational period it is proper to use a single antibiotic. In our initial experience with novobiocin in staphylococcal septicemia, the first 6 out of 7 patients were cured. When we reviewed the data, 4 of these patients also had a major surgical procedure performed, which no doubt helped. Then the next 3 patients did not respond to novobiocin alone. This type of investigation is necessary before we can make recommendations about combinations.

**Dr. Finland** (Moderator): Dr. Romansky.

**Dr. Romansky:** I think that as far as ristocetin, vancomycin, and kanamycin are concerned they ought to be used individually. The erythromycin, chloramphenicol, oleandomycin, and novobiocin group may be used in combinations. However, I think those first three (vancomycin, ristocetin, and kanamycin) certainly, at the moment, should not be used in combination with anything else.

**Dr. Finland** (Moderator): Dr. Yow.

**Dr. Yow:** I may have misunderstood Dr. Foltz, but I wonder why he thinks there might be organisms that will become resistant to these drugs (ristocetin, vancomycin, and kanamycin) in an individual patient, or that resistant strains will emerge in the general population. Is there any reason to suspect that that will be true?

**Dr. Foltz:** I am sorry that I do not have any adequate laboratory data to try and back myself up, but I have seen these cases of vancomycin treated staphylococcal infections in which we did not get cures when we were dealing primarily with a complicated septicemia and I think the addition of another agent is certainly indicated under those circumstances.

**Dr. Finland** (Moderator): This is not due to resistance of the strain involved, though, is it?

**Dr. Foltz:** Well, I say I cannot supply laboratory data on this. I know in certain of our infections the primary problem has been getting to the site of the infection, and I think your point is well taken that we have to consider the nature of the infection. Nevertheless, is it not possible that two agents might have a little different pharmacologic access to the site of infection, and is it not wise possibly to throw in another agent here if it is an active agent in the infection?

**Dr. Finland** (Moderator): Along these lines, I think, there seems a general agreement, with minor dissent, that vancomycin and ristocetin could be used alone but all the other drugs need support from other agents either for the prevention or the delay of the

emergence of resistance, or for increasing the bactericidal properties of the active agent. Now you have been discussing a number of useful agents, some of which must be used in combinations. Since these are representatives of many manufacturers in this audience who may value your opinion, or should have your opinion in any event, or others in this audience who look to you for guidance, I would like to pose this question: Is there any combination of the antibiotics that we have been discussing or any combination of these antibiotics with any others, that you would recommend to the manufacturers to combine in single vials, or single capsules, tablets, or other preparations. For the benefit of the doctors and their patients, are there any such combinations that you would like the manufacturers to produce for them? Dr. Yow.

**Dr. Yow:** Well, all I can think of, Dr. Finland, are some that I would like to separate.

**Dr. Finland (Moderator):** Dr. Romansky.

**Dr. Romansky:** Dr. Yow has expressed my sentiments.

**Dr. Finland (Moderator):** Dr. Foltz.

**Dr. Foltz:** No. I would like to see them used as required for the individual cases.

**Dr. Finland (Moderator):** None of the members of this panel knew that this question was coming and so you have their spontaneous and unanimous opinion.

Anybody who is interested has the opinion of these experts here. There is one more question that we might discuss before we close this panel. It happens that we here are all medical people, internists, and we are at a disadvantage in telling surgeons what to do. However, surgeons do, in some places request and accept the opinions of their internist colleagues with respect to certain aspects of antibiotic therapy, although they do not always follow the recommendations. This reliance on the internist for advice on antibiotic therapy is true at least of those surgeons who are not themselves intimately concerned with problems of antibiotics as a speciality, of which there are a number of very eminent surgeons in this country—many of them in this audience. However, we, as internists, are interested in their patients, as well as our own, and in the welfare of all patients. They have a right to get our opinions and advice. Therefore, I would like to ask the members of the panel how they feel about the prophylactic use of antibiotics in the management of surgical problems. Dr. Foltz.

**Dr. Foltz:** The cases in surgery in which I feel that prophylaxis against staphylococcal infection may be utilized with some justification are primarily those in which long operative procedures are involved, and particularly those in cardio-thoracic surgery certainly would justify the use of these drugs to prevent a catastrophic event after a long technical procedure. There are those cases, also, where inevitably an infection is encountered in surgery and on the basis of the infecting organism or information as to the probable infecting organism, sometimes prophylactic use of antibiotics is indicated. I think the eye surgeons certainly are also entitled to use local prophylactic therapy, because this is again another area where a catastrophic event may occur, if infection occurs after eye surgery.

**Dr. Finland (Moderator):** Do you have recommendations as to what they would use under those circumstances?

**Dr. Foltz:** I think this has to be decided on the basis of the local situation with regard to flora and sensitivity; I would urge that they use the most effective drug in full dosage rather than teasing the organism with suboptimal dosage.

**Dr. Finland (Moderator):** Dr. Geraci, do you have any recommendations to your surgical colleagues?

**Dr. Geraci:** No, except in cases in which there is a dirty field, say, where there is an arthritic infection and where you already know the organism. I think if you know the organism and the sensitivity you could give therapy beforehand, such as in cases of osteomyelitis where you are going to do a sequestration or where you are going to clean out a joint. In cases of heart surgery, I think we have recommended to our surgeons that they use penicillin and streptomycin in combination for prophylaxis and the dosages that we have recommended have been a million units of aqueous suspension of procaine penicillin every 12 hours, plus 1 Gm. of combined streptomycin-dihydrostreptomycin in conjunction with the penicillin for several doses before the surgery and for several days after the surgery.

**Dr. Finland (Moderator):** Are you keeping records of the responses? I hope that we shall someday have some data on whether this prophylaxis is really useful. Dr. Kirby?

**Dr. Kirby:** I would just like to raise the possibility that we may be entering a new era in regard to prophylaxis with the advent of bactericidal drugs to which staphylococci do not seem to become resistant. It seems well established that streptococcal infections are not what they once were because of the widespread use of penicillin to which streptococci have not become resistant. When penicillin was first available there were articles in the literature showing that when penicillin, and later penicillin and streptomycin, were given prophylactically following surgery, infections were prevented. At the present time post-operative prophylaxis is considered unwise because it leads to infections with antibiotic-resistant bacteria, notably staphylococci and certain gram-negative bacteria. I think the advent of vancomycin and ristocetin should at least call for new studies of this subject. It seems to me quite possible that small doses of these drugs, given for two or three days postoperatively, might destroy staphylococci that have entered the patient's body during or following surgery. If successful, a decreased number of staphylococcal pneumonias and wound infections would result, and this should be relatively easy to determine in hospitals where the incidence of such infections is high. This is sheer speculation, of course, but I think it might work, particularly if staphylococci do not become resistant to these new antibiotics after widespread usage.

**Dr. Finland (Moderator):** Are you recommending this to your surgeons?

**Dr. Kirby:** No, not so far, but we are hoping to collaborate with them in a study in which every other patient undergoing major surgery will receive vancomycin or ristocetin postoperatively.

**Dr. Finland (Moderator):** Dr. Quinn.

**Dr. Quinn:** I feel that the use of prophylactic antibiotics by the surgeon should be discouraged. It is difficult for a medical man actually to tell them what to do because they find themselves in some tight situations, but I think that we should encourage them to conduct controlled studies in which the answer to this can really be found. So far most of those types of studies have not shown prophylactic antibiotics to be of value.

**Dr. Finland (Moderator):** Certainly, would you suggest then, that Dr. Kirby get together with his surgeons and arrange some program from which perhaps, at some future time, we might learn whether his program is worth recommending? Dr. Romansky?

**Dr. Romansky:** To emphasize Dr. Quinn's point, certainly in the clean surgical cases, I do not think prophylactic chemotherapy should be used at all, and I would hope that if Dr. Kirby carries out what he anticipates that it be in a controlled fashion.

**Dr. Finland (Moderator):** Dr. Yow.

**Dr. Yow:** I certainly agree with the rest of the panel. I would like to add one comment along the lines of Dr. Kirby's statement. One of the most difficult types of infections to treat complicating surgical procedures are those that may be associated with the formation of hematomas or transudates that collect in the body cavity, and it may be quite difficult to get an adequate level of antibiotic into these areas to inhibit the organism, particularly if the *Staphylococcus* is present. Actually surgeons have been using this method but we are undertaking now a controlled study of local application of some bactericidal antibiotics such as bacitracin or neomycin into the operative site in procedures that are most likely to be associated with oozing of blood, like some of the orthopedic procedures or some of the cardiovascular procedures. This is just one-shot prophylaxis, in other words, at the site of the operation itself. These are procedures, too, where, if infection does occur it may be fatal. I mean, it does not take but one stitch abscess in an aortic graft to kill the patient, and it may be indicated under those circumstances.

**Dr. Finland** (Moderator): Does anybody have a last word of wisdom to impart to our listeners here? Dr. Foltz?

**Dr. Foltz:** Since I was the only one who spoke up for at least some instances of surgical prophylaxis, I would like to re-emphasize that prophylaxis should not be a substitute for aseptic surgical technique, and I think the surgeons are realizing this at the present time.

**Dr. Finland** (Moderator): Well, you have been very patient, ladies and gentlemen. I am sure that we could go on and discuss the problems before us for a long time. Perhaps, we did not generate enough heat in discussion and quarrels among our panelists, as some of you had hoped, but I hope that at least you have obtained some information from this group of experts that might be of use to you, whether you are physicians, surgeons, or perhaps even drug manufacturers. Thank you very much, all of you, and thus we will close this first panel.

# Causation, Prevention, and Control of Staphylococcal Disease in Hospitals

## Panel Discussion

**Robert I. Wise, Moderator**

### Members of the Panel

**Chester Howe**  
**George Gee Jackson**  
**Vernon Knight**

**Norman Learner**  
**Phyllis M. Rountree**  
**Thomas E. Shaffer**

**Dr. Wise (Moderator):** We hope to end on a high note at this late time and discuss the problem of staphylococcal disease, particularly the problem as seen in hospitals. At lunch, I tried my best to keep the panel members talking about things other than staphylococci, because their comments were so spirited I felt that they would lose their enthusiasm. We have eliminated many slides in order to make the discussions as brief as possible. I would like to introduce Dr. Chester Howe, of Boston, who will discuss the surgical aspects of staphylococcal infection; Dr. Norman Learner, from Philadelphia; Dr. Vernon Knight, next, of Nashville, and we are quite pleased to have Dr. Phyllis Rountree of Sydney, Australia, join our panel; Dr. Thomas Shaffer of Columbus is with us; and Dr. George Jackson of Chicago.

A 22 year old man came to the hospital with signs of a systemic infection that was thought to be a viral disease. He had a normal white blood count, and normal differential; examination of his chest was normal. His roentgenogram was normal. In three or four days, while in the hospital, he developed more fever, his white count increased, and his differential changed, and he was found to have changes in his lungs. Material from the pleural space was examined, and staphylococci were found to be present. An acute phase serum that had been collected prior to this roentgenogram and a convalescent phase serum were examined for antibodies to influenza A and indicated he had had influenza. This patient was treated with appropriate antibiotics and survived. This represents a hospital-acquired staphylococcal pneumonia in a patient with influenza. This occurred prior to the Asian influenza endemic.

Another patient, an infant, developed pneumonia while in the nursery. A large cyst was found in the right upper chest. This represents staphylococcal pneumonia in an infant, which was acquired in the nursery.

Another infection occurred recently in our hospital, It demonstrates an infection of a baby's breast with infections of the skin.

A 44 year old woman came to our hospital to have an incisional hernia repaired. She had had the hernia for a number of years. A few days after apparently successful surgery, she developed fever; her blood pressure fell to zero; she had a wet, cold, clammy skin and exhibited the picture of acute peripheral vascular collapse. She was having bowel movements quite frequently, and when measured they were about 600 to 1000 ml. in amount. She rapidly became dehydrated. A stain of her feces revealed myriads of staphylococci. This is not a frequent finding clinically, in fact in some four years I have only been able to confirm about 5 cases of staphylococcal enterocolitis, in a 1000 bed hospital.

This is the kidney of another patient who acquired an infection in a hospital. Bacteremia occurred and a large renal carbuncle was found present at postmortem examination.

We are not here to discuss whether the problem of staphylococcal infection is increasing or decreasing, but I think that we do have a problem of infection for which we need

to find ways of control and prevention and perhaps to treat more effectively than we are doing. The staphylococci were present in some balance in nature before we first developed antibiotics. Changes have occurred.

I have asked Dr. George Jackson to discuss the ecology of the human and the *Staphylococcus* in the community.

**Dr. Jackson:** Thank you, Dr. Wise. Our understanding of the ecology of the *Staphylococcus*, I think, has been very slow in developing and is still entirely incomplete. Some foundation has been set in papers preceding us this afternoon, and I shall try briefly to make two or three points that I think are important in our increased understanding of the ecology of staphylococci. First of all, it seems to me that the increase in knowledge has come from the development of techniques for following the identity of specific strains and their genealogy in the community. In the past decade investigators have used serologic typing, patterns of lysis with bacteriophages, and sensitivity to multiple antibiotics as more specific markers than we have previously had to trace the identity of staphylococci throughout the community populations. It is my impression from these data that the epidemiology of or epidemiologic spread of staphylococci is similar in many, if perhaps not most, respects to that of certain of the other endemic bacterial genera with which we are familiar. I think that a certain degree of valid comparison can be made with streptococci and pneumococci and with the enteric group of pathogens for which we already have the epidemiology quite well worked out.

The balance that exists between the endemic reservoir and clinical infections is a function of the triad of (1) the specificity of the strain, (2) the selective nature of the environment, and (3) the susceptibility of the general host population. I will try to document from our experience these three aspects of the epidemiology of staphylococci.

First of all, I think it is a mistake to think, or speak, as we often do, about the ubiquitous *Staphylococcus*. Instead, every isolate represents a specific strain that is moving into and out of the community and changing its base of prevalence from moment to moment. The specificity of the strain in a particular community under study becomes of utmost importance. Some of the strains unquestionably have increased virulence as compared to others. Some of the strains may be more likely to produce a specific type of pathologic lesion, or have special affinity for a certain tissue, thus we might have osteomyelitis, breast abscess, or gastroenteritis. I think this is comparable to our knowledge of specific nephritogenic types of streptococci. It might be analogous to the difference between higher type and lower type pneumococci, and to some of the clinical differences among the groups of *Salmonella*. As we learn more about the *Staphylococcus*, I think we will begin to unravel some of these differences with strains of *Staphylococcus*.

In the community, the most important selective environment or agent that we have found so far is the extent of the use of an antibiotic, or contact with it. I am not going to dwell further on that. You are familiar with it and perhaps it will come up for later discussion. There is still little information about the susceptibility of the host, but I believe that data are accumulating to suggest that also the general susceptibility of the host either with regard to previous contact with an epidemic strain or his general state of health, as with the other endemic infections, is of considerable importance in the production of clinical staphylococcal infections.

This afternoon you have heard a good deal about the carrier rate of staphylococci. In 1939, McFarland studied a group of healthy students in England and found that 34 per cent of that group carried coagulase-positive staphylococci. During the influenza epidemic last fall and this spring, we studied approximately 1000 patients. From this study there was no difference in the carrier rate among the patients, whether they were office or hospital patients, and indeed there was no significant difference between these patients and their well home contacts. Furthermore, there was no significant difference from the figures McFarland obtained a score of years ago, and that was well before any clinical use of antibiotics.

There is a great deal of difference among carriers. In previous papers today, the

number of staphylococci and the persistence of staphylococci have been mentioned but the point is that the *rate* of carriers is not necessarily a valid index of the extent or the severity of the endemic infection.

During the decade after the introduction of penicillin, everyone recognized the changing ecology of penicillin-resistant strains of staphylococci. I have compared our data from 1958 with the data we obtained in the same locale in 1952. It is clear that since 1951–1952 to 1957–1958, there has not been any change in the ecology of penicillin-resistant strains among hospital patients and outpatients. One might wonder then whether or not a dynamic equilibrium between penicillin-sensitive and penicillin-resistant strains has not been reached and the level of that equilibrium for hospital patients is about 75 to 85 per cent resistant strains and for the community something like 25 to 35 per cent resistant strains.

This differs, of course, with different antibiotics, but I think that with each of them the same phenomenon has been demonstrated to a greater or lesser degree and there is a different level of equilibrium established in the hospital and among different community populations. Antibiotic-resistant infections are still predominantly centered around the hospitals.

The phage-typing experience in our laboratory for the past 7 years or so, demonstrates a point or two that I would like to make about the survival of strains or perhaps resistance among the host population. In 1952 more than a third of the strains that we typed were of the single phage type 42B/47B/47C. In the subsequent years in one locality, there was a cyclic and rhythmic decrease in the frequency of this strain, until in 1956 we did not recover a single isolate of this particular phage type. During the same period of time, another strain 3A/3B/51 showed a similar but reciprocal rhythmic cyclic change in the community until in 1956 this strain represented approximately 35 per cent of our typable strains. Thus, I think, that with strains of staphylococci, the ingress and the egress in the community is similar to that with other specific bacterial strains, and from our data one might suggest a cycle of 5 to 10 years for ingress and egress of a particular epidemic or endemic strain. I would like to speculate on something the data might suggest. For one strain the peak incidence in each of the cycles occurred during the winter months. On the other hand, with another strain the peak incidence of the cycles tended to occur during the summer months. One can wonder whether or not seasons play some important role in the ecology of the *Staphylococcus* as with some of the other bacteria and viruses.

Similarly, as Dr. Wise suggested, during the years up to 1955, we were frequently bothered with the syndrome of staphylococcal dysentery or gastroenteritis commonly associated with the prevalent strain. During the past three years since the disappearance of this strain we have had almost no staphylococcal gastroenteritis. It may be therefore that there is some affinity for certain tissues or preponderance for a type of lesion among specific strains.

In summary, I have tried to present the thesis that staphylococci in the community are not ubiquitous and uniform. The accumulation of data by methods that identify specific strains indicates that specificity is as important in the ecology and epidemiology of staphylococci as it is in other types of bacterial infections. Thus, the frequency of staphylococcal carriers in the community may not be a reliable index of the problem of staphylococcal infections. Environmental factors, especially use of antibiotics, influences the selection of strains within population groups. In addition to the specificity and selection of strains in the community host factors such as age, health, immunity, and respiratory infections, which influence the number of staphylococci harbored in the nasopharynx are undoubtedly of major importance in the causation and control of staphylococcal infections.

**Dr. Wise** (Moderator): Thank you, Dr. Jackson. As I listened to Dr. Jackson, and heard Dr. Walter this afternoon, I was reminded of the work that was done some years

ago. When a patient was placed in a sterile chamber, and sat quietly and samples of air were examined for bacteria, they were found to be low in number. As the person turned slowly more bacteria were present in the air. If he exercised energetically many more bacteria were found, particularly staphylococci. I picture as a result of this study people walking down the streets in the community, exchanging their staphylococci by walking through each other's clouds. As a patient enters the hospital, new selective forces are applied. We have evidence that the ecology in the hospital is different from that in the community, which Dr. Jackson has described. Some excellent studies have been done in hospitals. Changes in the bacterial flora of the patient occur during hospital stay. Dr. Vernon Knight will discuss this aspect of the problem.

**Dr. Knight:** Dr. Jackson and others have described the rise in resistance of staphylococci to various antibiotics as these drugs have been introduced, and, since hospitals are the commonest place where drugs are used, there has been a resulting concentration of resistant staphylococci in these areas. Many other people have observed this occurrence and have described its various features. It has been discovered that a *Staphylococcus* that becomes resistant to one drug is apt to become resistant to another, so we find in hospitals a large proportion of strains that are multiple-resistant. Further, it has been found that these strains are generally lysed by group III phages, or phages 80 and 81.

When a new patient is brought into a hospital filled with drug-resistant staphylococci, and he carries staphylococci that are drug susceptible, we have found he will acquire resistant strains more or less rapidly depending upon the treatment or no treatment he receives in the hospital.

Thus we observed that most strains of staphylococci carried by new patients were susceptible to tetracycline and those in the hospital were resistant to it. When patients were given tetracycline, a very rapid change occurred, often within hours, in which resistant strains replaced susceptible strains in the new patients. New patients carried strains about one half of which were resistant to penicillin and these patients failed to lose these strains when given this drug, so that the rate of replacement in patients given penicillin was slower than with tetracycline. Untreated patients had no impetus to change strains and replacement occurred least rapidly in them.

These studies were all made in hospitals in which a reservoir of resistant strains was already present, but in the past year we have studied patients at a mental hospital where antibiotics were seldom used and the staphylococci were generally susceptible. We found that staphylococcal carrier rates of patients given penicillin reduced, but by the second week resistant strains appeared that arrested the fall in carrier rate at about the mid-point. After treatment was stopped, resistant strains diminished and the carrier rate climbed back toward the pretreatment status.

Tetracycline produced about the same changes, except that carrier rates were initially reduced to about 20 per cent by the treatment. We further found that the use of penicillin followed by tetracycline caused the disappearance of all of a large number of penicillin- and tetracycline-susceptible, 52-80 strains, and this was associated with a large increase in phage non-typable penicillin- and tetracycline-resistant strains—in a small way reproducing a reservoir of drug-resistant strains, such as have been described earlier today.

These studies have suggested to us that programs of restriction of use of common antibiotics in the hope that they will significantly restore their effectiveness in treatment of staphylococcal infection is not a very sanguine one.

**Dr. Wise (Moderator):** As I listened to Dr. Knight, I could not help but wonder whether the 80/81 strain really is more pathogenic than the strains that Dr. Jackson talked about in the community. If we can assume that the 80/81 strain is more pathogenic than others, and I am not sure that this is true, and there is selection in the patient of these antibiotic-restraint strains, does the patient who receives antibiotics

have more infections than a patient who does not receive them? Does an antibiotic stimulate infection in a patient? I am going to pose that question and perhaps have it discussed later. The patient in the hospital may go to surgery, and there other things happen to him. Dr. Chester Howe will discuss briefly, the surgical aspects of this problem.

**Dr. Howe:** Perhaps the best I can do in the time allotted to me is to make a few remarks that might orient some of the people who are not in the surgical field to a surgeon's point of view. One important concept or principle is that the use of antibiotics in the treatment of chronic suppurative disease, or staphylococcal disease once it becomes localized, should be considered as adjunctive to surgery and not as a curative measure. One of the most frequent mistakes one sees made is persistent, fruitless reliance upon antibiotic treatment when you need surgical drainage.

From here on I would like to limit my remarks to clean wound sepsis. Although there is no proof for it, there is considerable evidence to suggest that most of these clean wound infections are seeded in the operating room and not on the ward. There are a number of reasons for this. First, the time relationship between the operation and the discovery of the infection, which is usually about the fourth to the seventh day. There are some delayed cases, but by and large the sepsis is apparent at the first dressing. The second reason is that if you open up enough of these wounds you will notice that the central focus of the infection is deep, not superficial, as you might expect if it was ward acquired. The third point is the frequent association of these infections with hematomas and lastly the well-known difficulty in initiating experimental wound infection in an animal after 48 hours has elapsed. I do not believe, and I think most of my surgical colleagues will agree, that we frequently see a serious staphylococcal infection develop in a closed wound on the ward. I am not talking about burns, and you may get some low-grade infection around drain sites, but they are not the serious life-endangering major infections, which are the real problem.

Now, just a few words about what the surgeon can do to prevent infection. If I had to pick out one most important factor, in the prevention of staphylococcal disease, it would be excellence in surgical technique. By this I mean accurate, gentle handling of the wound, accurate hemostasis, and avoidance of dead space and hematomas. Wounds that are gently closed without dead space and without tension, with good hemostasis, seldom become infected, despite the fact, that they probably are all contaminated as Dr. Wise and others have shown. Dr. Wise has a study which is not published yet, which shows that about 60 per cent of clean wounds had staphylococci in them as demonstrated by saline washings at the end of operation.

Next in line, comes a tightening up of all aseptic and antiseptic techniques relative to sterilization in the operating room and on the wards. This is a big field, and I will not try to go into it; Dr. Walters has discovered some of its aspects in his paper this morning. I would like to emphasize isolation. I think we all must try to isolate these serious staphylococcal surgical infections that are draining pus, and practice good barrier technique. I know this constitutes a problem in many hospitals where you cannot isolate patients, but at least you can isolate the wound with an occlusive dressing and stop the pus from getting into the bedding, to dry, and becoming air-borne. Another point is that a surgeon must be concerned with all types of staphylococci whether it is an 80/81, or some other type. The main criterion of the virulence of an organism is whether it can set up an infection in a patient. We see wound infections with other than 80/81 strains that are serious.

Now above and beyond these things, there exist many exercises in environmental disinfection that in some instances seem reasonable, but for which there is no proof of a favorable effect on the infection rate. Sterilization of blankets, the air, mattresses, and floors fall into this category. It is easy to get data to show that a particular technique lowers the number of organisms in a certain phase of the environment. What

we really need are data that show the effect of these various techniques on the infection rate. We need to know not only the importance and the virulence of various strains of staphylococci, but the importance of various reservoirs of staphylococci. We must remember the work at Great Lakes Naval Station carried out by Capt. Seal and others where they studied environmental disinfection of blankets and air. They succeeded in lowering the air counts and the blanket counts, but this had no appreciable effect upon the infection rate, even though they controlled to a certain extent their environmental factor. True, they were studying streptococcal disease and maybe staphylococci will act differently. It seems to me that the bulk of evidence relates the spread of staphylococci to people more than to the environment.

**Dr. Wise (Moderator):** Thank you, Dr. Howe. A bacteriological analysis of 54 surgical wounds was performed. Just before the wounds were closed, we asked the surgeon to put in 5 ml. of sterile saline, wash the wound, and withdraw 1 ml. This was cultured quantitatively. Only 20.3 per cent of the wounds were sterile; 20.3 per cent contained other organisms; and 59.3 per cent of the wounds contained staphylococci, of which 28.1 per cent of the wounds contained coagulase-positive staphylococci. Of these 54 wounds, only 3 developed infections. I want to emphasize what Dr. Howe has stated, that is, bacteria including coagulase-positive staphylococci are present in surgical wounds.

Now, we shift to another part of the hospital, one that has received a great deal of attention, one in which the human exists in a state of great susceptibility, and one in which, perhaps, there are certain factors that increase the density of certain types of staphylococci. Dr. Thomas Shaffer will discuss the nursery and its problems.

**Dr. Shaffer:** For the benefit of those of you who are not accustomed to hospital nurseries, I think I could speak for all pediatricians in saying that hospital nurseries are not now, and never will be, ideally safe places for babies. A study by J. L. Henderson, of Scotland, performed before antibiotics were available indicates that back in 1942 as well as in 1958, after the first week of life, infections were a serious problem. This is probably typical of the situation as it will always be regardless of what we can decide here today.

Mothers must stay in the hospitals, however, because it is the best place for them and so the babies must stay, but I am sure that if the babies could be sent home after the first 24 hours, after we were sure that there were no complications from birth injuries, asphyxia, and congenital anomalies, the entire hospital staff would breathe more easily. There are many reasons for this: babies are little, and it is easy to get a lot of them in a small space; people are needed to take care of them so that nurseries are unusually crowded, compared to other areas; babies apparently are more susceptible to colonization by staphylococci and perhaps to infection, and, therefore, they are very likely to pick up infections. We are using more and more non-professional personnel in hospitals, and many of them are not so convinced as those of us here about the benefits of good technique. I think that even doctors and nurses have become a little careless with antibiotics available, and good technique has gone by the wayside in many instances.

About 12 years ago in England, later appearing in Canada, in Australia, the United States, and finally in the rest of the world, there have been widespread virulent staphylococcal infections in hospital nurseries, affecting mothers and babies. Apparently, from the experience of those who have been working among newborn infants many years, this is a new situation compared to 30 or 40 years ago. A lot of information has been gathered during the past 10 years about these staphylococcal outbreaks, and certain facts have been substantiated; it is about those that I wish to speak at this time. One fact is that there apparently is a clinical entity of staphylococcal disease in the neonatal period that in epidemics is manifested by a spectrum of symptoms. On one end of the spectrum being conjunctivitis as the mildest manifestation, going through

other visible manifestations such as pyoderma, then to omphalitis and cellulitis, (mastitis in the newborn infant, in the experience of all pediatricians I have spoken to, is a most unusual occurrence except in an epidemic in nurseries) and then pneumonia, empyema, and osteomyelitis.

This, I think, corroborates what Dr. Jackson has said, that there seems to be some specificity among different strains and among infants this seems to be more marked than in adults. Secondly, in an epidemic in the nursery there is a predominance of one particular phage type of *Staphylococcus aureus*, and in most of the epidemics throughout the world this has been phage type, 80/81. In our experience, when random samples have been sent to us for phage typing, from outbreaks in which the phage type was not known, 9 times out of 10 strain 80/81 was the cause of the epidemic. Just as a rule of thumb the odds are 9 to 10 you have a hot strain if you have breast abscesses among mothers and infants and increasing evidence of infection among babies. Thirdly, nasal colonization of the babies occurs prior to clinical infection. There is often a prolonged latent period, which may exceed the infant's hospitalization, between nasal colonization and manifest infection.

To illustrate this, in the nursery during an epidemic, we cultured the nasopharynx of 74 infants and found that 24 had been colonized by the 80/81 strain by the time they went home, illustrating the susceptibility of the babies to colonization. However, only one of these babies became clinically infected while he was in the hospital but 16 more had clinical infections after discharge from the hospital. This situation makes it mandatory that in a hospital we keep some sort of surveillance of the community, particularly among postpartum mothers and recently discharged babies; otherwise, one may well not be aware of epidemic conditions in the nurseries. This might be done by questionnaires which mothers take home for return a month later. It may also be done by telephone calls as has been suggested. Once these babies begin to develop infections at home, there is a tendency for the organism to spread within the family, and this may be experienced during a period of three to six months after the babies have been discharged from the hospital during an epidemic. A series of families were surveyed in a small town in Ohio three to six months after infected babies were discharged from hospitals and three quarters of the mothers were carrying the epidemic strain and almost half of them had had lesions. Fathers were colonized to a lesser extent (28 per cent) but a great many of them had lesions, and even many of the siblings now had the disease. It is not hard to imagine where the reservoir of infection by strain 80/81 is coming from if one realizes that the community is being seeded, or has been seeded, many times from hospital epidemics, many of which were not recognized at the time.

Another point of interest is that mothers are probably not the source of the hospital epidemics but hospital personnel and infected patients themselves are unquestionably implicated. I think that in time we will have to worry about mothers as sources of infection because of the 72 per cent of colonized mothers already mentioned, many will return within a year or two to have another baby. Apparently the close contact of nurses with babies is the important factor in initiating an epidemic at the present time.

There is some benefit in controlling known carriers of the epidemic strains in the hospital. Our personal experience in the past year, during which time every person who works in our hospital nursery, or on the maternity floor, is cultured before being assigned to the floor, has been good. All personnel working in the maternity unit are cultured every month, and nasal carriers of the recognized epidemic strains (in our community we now have five, where we used to have only 80/81) are refused assignment in that area. They are given other assignments if they pick up the organism. During a period of 12 months we have had an infection rate of less than 0.5 per cent among 4000 babies, and only 2 babies out of 4000 have had infections due to epidemic hot strains. So, there is some benefit in finding these asymptomatic carriers.

Finally, one might say that some measures to prevent the contamination of the environment by colonized babies is worthwhile, and I present to you the data of Cunliffe, in England, showing the daily increment in percentage of babies who had *Staphylococcus aureus* in their nose so that at the end of six days 100 per cent of the babies showed colonization, by coagulase-positive staphylococci. This has been reported repeatedly in various parts of the world. Our experience, after adopting a policy to wash the vernix from the babies immediately upon admission to the nursery and every 48 hours thereafter appeared to show that cutting down on the air-borne spread of very light dried vernix decreased nasal colonization to 30 per cent of the infants in six days. We think that in this way we have controlled the spread of organisms from baby to baby. These are known facts. Their relative influence in epidemics and application in the final answer to the control are matters that have not yet been fully determined.

**Dr. Wise** (Moderator): There are a great number of questions, of course, that arise as a result of this discussion. Why have these organisms been selected in this environment? What part do the antibiotics play in this selection? Some would think that antibiotics have played no part, others think that perhaps they are all-important in this selective process. What is the importance of the carrier? Some people have stated that carriers are unimportant. Others state that the carrier is all-important. What should be done with the carrier? At Temple Hospital there have been early efforts to have team participation throughout the hospital in the control and prevention of infections. Dr. Norman Learner will discuss briefly the hospital committee and efforts at control by hospital personnel.

**Dr. Learner:** When early in 1956 at Temple University Medical Center we noted a sharp increase in the incidence of staphylococcal infections in clean wounds and in hospitalized patients and personnel, certain control measures were instituted. We feel that these steps have been responsible for a more recent marked decline in these infections. Initially, we established a Committee on Infections. Most of the personnel infections occurred in student nurses who had the most intimate contact with patients. In the student nurse, the site of these cutaneous infections are mainly on exposed surfaces. Sixty-four per cent of infections involve the forearms, hands, and face, all areas readily exposed to contamination while in nursing uniform.

In recent years there has been a relaxation in hospital and operating room techniques; a return to strictest precautions is essential. Some of the control measures taken by us are outlined.

Hospital personnel with cutaneous staphylococcal infection were taken off duty promptly. Strict isolation of all patients with staphylococcal infections would be ideal. Since this was impractical, a modified isolation technique was developed in which all bedding and clothing in contact with the patients were placed in separate, plainly marked bags before transportation to the laundry. Patients on whom surgical procedures were planned were not placed in semiprivate rooms with patients having staphylococcal infections. Spot checks of blankets revealed a number of positive cultures of coagulase-positive staphylococci. Due to rapid turnover of patients, it was found that blankets were not being laundered after individual patient discharge. This was corrected. Hospital cleaning methods were intensified and wet mopping was substituted for dry sweeping wherever possible. Soap and water and 70 per cent alcohol were placed at bedsides of infected patients. Hospital personnel used these as a hand rinse after any contact with the patient. The practice of wearing operating room clothing and shoes outside the operating room was forbidden. Filter-type operating room masks replaced the multiple-layer gauze type that had been used previously. Caps and masks were changed between operations. Many other operating room techniques were modified.

Indiscriminate use of antibiotics, particularly as prophylactics pre- and postoperatively, was discouraged. It was recommended that those systemically administered anti-

biotics to which our phage type was susceptible be withheld unless sensitivity studies and severity of the infection warranted their use.

In conclusion, successful control cannot be obtained by new antibiotic agents alone, but must be predicated on strict, sterile techniques, combined with intelligent limitation of the use of antibiotic agents.

**Dr. Wise** (Moderator): Thank you, Dr. Learner. Our Committee, in our hospital, has been functioning now for a few months. The group that has done most in our hospital has impressed me with one thought. We divided our Committee up into six study groups. There is one group for the study of the operating room; there is another group for the study of the nursery; another group for the study of the handling of infected patients and septic materials; and another group studies therapy and makes suggestions with regard to the withholding of antibiotics or the use of certain antibiotics; and there is another group devoted only to the study of the physical plant and the house-keeping practices. As you see, this plan is modified after the report of Dr. Ellard Yow and the plan that was used at the Jefferson Davis Hospital to control their problem of infection. The group that has made most progress in our hospital has been the group composed of people who are studying the physical plant and housekeeping practices. On this Committee we have the housekeeper and her assistants, the maintenance foreman of our building, meeting together with surgeons, obstetricians, pediatricians, nurses, and so on. I would certainly add one note of emphasis. In the hospitals which are organizing their Committees, it is not well to have too many generals, but remember those people who actually do the sweeping and scrubbing. If people sit together once a week and discuss their problems, and the principles are not taught to the grass roots people, I do not think the program will be as effective. Within one week after our first meeting, our housekeeper had made many changes. She now has seminars in her own department for her personnel who work at keeping the hospital clean.

I have asked Dr. Rountree to say anything that she wishes. I would like to hear what she will say as a result of her recent trip to England, and also what she may see in Australia that is different from our problem in the United States. We will be glad to know if she has any predictions or advice for the future. Dr. Rountree.

**Dr. Rountree:** I have only two points to make at this stage in the discussion. The first is, we must remember that staphylococcal disease is an infectious disease. This is most important. No one would ever dream of leaving a case of typhoid fever in a medical or surgical ward. However, day by day we see patients whose surgical wounds, respiratory tracts, and perineums are releasing large numbers of staphylococci and still they are allowed to remain in the wards. Now, Dr. Learner has already mentioned this and has said it is not always possible to isolate. Well, I say that if this was a patient with typhoid fever one would not say it is not possible to isolate. Until one knows the actual infection rate in a hospital, until one treats this disease as an infectious disease, we really will not get very far with its control.

There are one or two other factors that must be considered, too, in considering the seriousness of the problem. They have been touched on already by the other speakers, and they include the fact that we do think that there are new strains, which have appeared recently, that may be different from older ones. These may have high infectivity rather than high virulence, but certainly they are able to spread. These are the strains that have caused the postnatal infections, in Australia, in Canada, in America, many parts of Europe, and in New Zealand. At present, apparently as far as I can determine, these strains are only hospital strains in America, but we have already seen them spreading into the community. Well, as far as I can see, Australia has perhaps two years start on you in this respect, and what is happening to us now is perhaps

TABLE I

*Annual Incidence of Generalized Staphylococcal Disease  
Patients Admitted with Infection*

Year	No. of cases	80/81 strains		Other types*	
		No.	Deaths	No.	Deaths
1950	2			2	2
1951	2			2	0
1952	2			2	2
1953	2			2	1
1954	3			3	1
1955	4	2	1	2	0
1956	16	13	7	3	2
1957	15	8	2	7	1
Total	46	23	10	23	9
Per cent			43.5		39

\* Other types: 22 phage patterns, 1 not typable in 1954.

the basis of what I might make a prophecy about. I just want to draw your attention to table I. These are data from a series of patients in our hospital over an eight year period. Those patients who acquired their infections in hospitals are the ones we are worried about now and are discussing today. The other patients come into hospitals with staphylococcal septicemia. We had an average of two each year until 1955, then we had the dissemination of type 80/81 strains. Since then there has been a vast increase in the numbers of cases with staphylococcal septicemia. We hope that this will not happen here, but I understand that it has already happened in some other areas of the world. Their problem has gone out of the hospitals into the community and it is now coming back in to the hospitals again.

I just want to make one further point about this spread in the community. We are now making surveys of the incidence of soft-tissue infections throughout the continent of Australia. These are patients seeing doctors in their own offices, and 45 per cent of all of these infections observed are due to penicillin-resistant staphylococci. Thirty-five to 40 per cent of the infections are due to type 80 strains. Now this is really different from the figures that we have already seen this afternoon for carriers in certain selected groups of people in America. Further, it looks to me as if you are just at the beginning of your staphylococcal problem. Now I hope that discussions this afternoon will bring out some of the points on which we should concentrate in order to control these infections.

**Dr. Wise (Moderator):** Thank you, Dr. Rountree. In summary, there is a dynamic equilibrium in our community with many different types of *Staphylococcus*, going through various cycles. Many of these cause infection, and the majority of strains are susceptible to antibiotics. However, there is an increasing incidence in the community of strains that are resistant to the older antibiotics. When patients enter the hospital, there is a selection of strains that are more resistant to the older antibiotics, whether they are more pathogenic or not is something to be discussed. In various parts of the hospital, there are problems that depend upon factors that are peculiar to that part of the hospital. In the operating room where there is trauma to the patient there is opportunity for invasion by these opportunists, the staphylococci. In the nursery, there is a collection that is maintained. Perhaps this medium of new babies is inoculated by a carrier; staphylococci may remain there and be transferred from one to the other. Babies go home, become infected, and their mothers develop infections of their breasts. The home then becomes an island in the community, which for months or even years may harbor these strains and infections may be traded back and forth. We see re-

peated infections in mothers, fathers, and siblings. There is return of these resistant strains with perhaps increased pathogenicity back to the community. There are many aspects of this problem for discussion. Now, we would like participation by the audience. I would like to ask one question to start this discussion. Dr. Rountree, is there an epidemic strain? If so why do you think it is an epidemic strain? If we do have such a strain, is it more pathogenic than the other strains in the environment?

**Dr. Rountree:** Well, I would say that there are many epidemic strains but at the present time we are seeing a global epidemic with one particular strain. I do not say it is the only one, and I do not say that is the only one we will ever have. Why should it be regarded as an epidemic strain? It seems to have some characteristics that allow it to produce lesions on normal skin. It also is at least a penicillinase producer, which has allowed it to be selected by a hospital environment. I think these two factors are quite enough to account for it being an ecological success at the present time.

**Dr. Wise (Moderator):** Dr. Rountree, we have studied in the last two years a closed environment containing 300 people. They have had an epidemic; in the beginning there were 10 infections; two of these were caused by the 80/81 strain; and there were four other strains causing infection. In the next two years there were many infections and a number of deaths; 100 per cent of the abscesses that we cultured after the second month were the 80/81 strain, and yet during the entire two years there were approximately 26 different strains in the environment. After antibiotics were started, there was improvement in the abscesses, but the carrier rate increased. Perhaps Dr. Knight would like to discuss this, too. What in your opinion are the effect of the antibiotics on the carrier rate, and do antibiotics increase the possibility of infection in some patients?

**Dr. Rountree:** I think you have posed a difficult question. I do not know the answer.

**Dr. Knight:** I do not know the answer, but I would like to talk about it. I have been impressed with the data shown here this afternoon, that these nursery discharges were implanted with an 80/81 strain; they went home and then developed the infection—in an area where I would presume they are largely not receiving antibiotic treatment. Dr. Wise's institution, which I suppose is a mental institution, where probably the incidence of acute disease requiring antibiotics may be low, has been the site of a continuing outbreak of endemic or epidemic character. We see widespread occurrence of disease in homes where antibiotics are not being used. Thus it appears that the propagation of the disease depends on some character of the *Staphylococcus*, perhaps developed in that particular *Staphylococcus* as a consequence of exposure to drugs previously. Important to me in this problem is that the antibiotic therapy is not accompanying or apparently pushing the epidemic along at the time it occurs. This seems to me to be a worthwhile point to make.

**Dr. Wise (Moderator):** I would like to ask another question. I had not considered a point that was made by Dr. Walter recently. He did not think that we should call this an epidemic strain. Perhaps we should speak of it as an epidemic of decreased attention to certain techniques, Dr. Walter? Then Dr. Walter a little later mentioned an epidemic that he had observed, an outbreak in a housing group. Would you take a moment to tell us your thoughts about this?

**Dr. Walter:** This involves an enclosed community of graduate students, some hundred families, 500 individuals, living in a geographically circumscribed area of substandard housing. The two infants involved returned home to this community and an outbreak occurred involving about 100 individuals with skin sepsis. The data that I am quoting are still raw, but it looks as though we have baby sitters, pre-school nursery,

and a laundry firmly implicated in spreading these bacteria from the 2 infants and their families to the rest of the community. Now this may be an epidemic if you want to call it such, but it seems to me that it is more or less predictable transmission of disease from one typhoid case, for example, throughout a community. This is an epidemic, truly enough, but it is just an expression of infectious or communicable disease. We are not used to thinking about staphylococcal disease as being infectious, and I think that once we recognize this and treat it as though it were smallpox, typhoid, or diphtheria, our problem will no longer be as severe.

**Dr. Wise (Moderator):** Are there any questions that anyone would like to ask the panel from the floor. Dr. Waisbren?

**Dr. Waisbren:** A problem we all have on our infection committees is the question of carriers in certain areas. I would like an expression from the panel, as to how they feel about type 80/81 carriers in the operating room, nursery, and on their general medical wards? Do they allow them, should carriers be there, and what do they do with their operating room nurses and nursery nurses who are carriers?

**Dr. High:** I would just like to add to Dr. Waisbren's question. All I want to know is what you do with other members of the hospital personnel other than the poor nurses. What is done with a surgeon who is a carrier?

**Dr. Wise (Moderator):** Dr. Shaffer, what should be done with carriers?

**Dr. Shaffer:** This was a much easier question to answer about a year ago when there were not so many carriers. We now find it more difficult to answer because they are turning up every day. We do not believe that a carrier of a known epidemic strain, one that has caused disease in babies, should be in the nursery. We have a strict feeling about that. However, since in our experience and in others, during epidemics, the symptomatic carriers have been practically all limited to the nursing personnel in the nursery and have not implicated the anesthetist or nurses on the floor, we have not insisted that these carriers remain off the maternity floor. We have permitted them on the postpartum area. They are not permitted in our hospital to work in surgery. The medical people, internists, have no fear of this disease because, as it has so often been pointed out, the internist is not so familiar with the problem because he just does not see it, except in old or debilitated persons.

I have the greatest respect for the 80/81 carrier and I feel that wherever carriers are going to be working with susceptible patients they should be controlled.

**Dr. Wise (Moderator):** Dr. Knight, what is a carrier? We have been talking about carriers and you have been doing quantitative studies of carriers. We say that about 70 per cent of people have staphylococci. Would you discuss this point? I think it is important.

**Dr. Knight:** We *talk* about carriers. I think a lot of unnecessary misconceptions have arisen because we have, depending upon the method used, somewhere between 30, 40, or 60 per cent of the general population, wherever you meet them, in or out of hospitals with coagulase-positive staphylococci in their nose. Their skin will carry a high proportion, and they are usually the same phage type. They may be in the rectum or throat but this is not so common. Generally speaking, a person who is a carrier has the same kind of *Staphylococcus* widely distributed over his body surface. Now, from the point of discussion of staphylococcal disease, most people when they talk about the carrier, are thinking more specifically of a person who is carrying a drug-resistant strain, very often of the 80/81 variety that Dr. Shaffer is referring to. I think

this afternoon most of the time we are referring to an 80/81 carrier when we are talking about the carrier or the carrier of the epidemic strain. It is obviously impossible to contemplate and eliminate 50 per cent of our population from any particular pre-determined area for any long period of time. You could not operate a hospital if you eliminated all staphylococcal carriers.

**Dr. Wise (Moderator):** Dr. Jackson.

**Dr. Jackson:** In the spread of staphylococci, it is likely that persons who transmit infection most readily are those who harbor large numbers of staphylococci as has been shown conclusively to be the case with streptococci. In a recent study we showed that the proportion of persons with large numbers of staphylococci in the nose, 1000 to 100,000 per culture specimen, was significantly greater among hospital patients than among office patients. The same trend was apparent in throat cultures. On the other hand, office patients with a respiratory infection were no different in this respect than hospitalized patients with a respiratory infection.

**Dr. Wise (Moderator):** I would also like to emphasize this aspect. Two hundred and six people were met at the door of an operating room theater by Dr. Frank Sweeney or me. We obtained nose cultures of everybody who entered this hospital operating theater. We found that the carriage of staphylococci varied from 61 to 88 per cent, and the carriage of coagulase-positive staphylococci varied from 30 to 66 per cent. It is indicated that those that were closer in contact with infected patients harbored more coagulase-positive staphylococci. When we studied the infections that occurred two months prior and two months after this survey, we found five strains causing infection. Those five strains were only carried—at least we were only able to isolate them—from 12 per cent of these 206 people. In any survey, one must be sure of the definition of a carrier. Whether this is just a carrier of coagulase-positive staphylococci that are not causing infection or whether they are hazardous spreaders of infection is important. However, in recent discussions with some people, particularly pediatricians, I have heard the opinion expressed that when one does careful physical examinations on all the personnel who come into the nursery the most dangerous carriers are those with infections. Would you agree with this, Dr. Learner?

**Dr. Learner:** It has been our experience certainly that in outbreaks of spontaneous abscesses that appear on the wards, for example, that it is usual to isolate or determine that one of the nurses on that particular floor has a skin infection, particularly a boil on the hand, or some exposed part of the body. We found a higher correlation between that type of carrier and transfer to a patient than between the nasal carrier without any skin infection and similar transfer.

**Dr. Rountree:** I would like to make a further point about this. We have found definitely that people with type 80 in their noses nearly always have a history of lesions as well within a period of time. Eighty per cent of carriers so identified among their nursing staff had this history. And there is another practical point that arises from this. I understand you are going to do phage typing in an enormous way here so that everybody who wants to can have all their staphylococci phage typed. In some countries this is not possible, and so one has to have a rough guide as to what carriers to eliminate and what to leave in nurseries, for example, plus all operating theaters. We have always taken the view that has already been put forth here, that people with a history of recent lesions are the people who must in the first case be regarded as very dangerous.

**Dr. Wise** (Moderator): A practical point that comes up, Dr. Rountree, is that to phage type cultures of staphylococci is a laborious business. We have counted the hours that it takes in our laboratory and calculate that one-man hour is required to type two strains. Is this too slow? And when we do our typing, we sometimes are one to three months behind our survey. Since the patients change occasionally and acquire new strains and people who are not carriers become carriers, I have been hesitant or dubious about discharging carriers or doing something with them if we know they are carriers. In a population of 200 people if you get rid of the 24 that are carriers, I would think that in two months there would be 24 more. Would you think this is correct? Can one by shifting the carriers around do anything to control?

**Dr. Rountree:** Well, I think there are two points here. We are really talking about controlling—over-all control of a section, and what part the individual carrier plays in this. Now I do not think it is any good removing your carriers as long as you have got a dirty hospital.

**Dr. Knight:** I would like to make a provocative comment, which may arouse a response from Dr. Howe and perhaps others. I have been impressed that surgeons really have been accepting guilt for a lot of the business that is going on with wound infection; they have all assumed that in the good old days they were very clean and neat in their work and that they were aseptic in their approach. I was a medical student not many years ago with Dr. Walters, and I know he was one of the most rigid people about this. I also think that, although precautions may have slipped, I doubt that the increase in resistant staphylococcal infections in many hospitals can be directly attributed to slipping techniques. My concept is that we do not yet have techniques that are adequate for present purposes. I am hesitant to let the surgeon assume all of this guilt. We are probably at a point where we really need to go over the whole business to improve, by engineering and other procedures, ways of preventing spread of disease, rather than to say that we should go back to the good old days, because I do not think that they were that good.

**Dr. Wise** (Moderator): We have carriers present in about 6 to 12 per cent of hospital personnel. These people may be important in spreading infection. The important thing is to clean up our techniques and make them very rigid, to practice good surgical techniques, practice good techniques on the ward, and not use prophylactic antibiotics when they are unnecessary. We can dispose of this problem of carriers at this time. Are there any other questions?

**Dr. Howe:** I would like to say something about this. To get back to this question of carriers, I do not think it makes any difference unless you have an outbreak. Supposing you have an 80/81 carrier but you have no outbreak. I do not think you have justification for putting that person out of the operating room. If you do have an outbreak, it will be as Dr. Rountree says. In the majority of instances it will be the same person who has a lesion or a history of a lesion, so there really is no problem. Now the majority of our hospitals, Dr. Rountree, do not have access to phage typing. Furthermore I do not think that it is indicated to advise all hospitals routinely to phage type their people to see whether they are carriers of the 80/81 strains. So the problem is really not so difficult as it seems. It amounts to this—if you do have a research problem going, you know it—if you do not have an outbreak I do not think carriers should be taken off duty or penalized in any way. If you do have an 80/81 carrier, then in an outbreak, he definitely should be taken off duty. In regard to this question that has been posed by Dr. Knight, I agree. I think that the use of antibiotics has caused a subtle deterioration of techniques, hard to discern among some surgeons, and I

think it has caused a subtle deterioration in diagnostic acumen among medical men in covering up diseases by the premature use of antibiotics. At the same time, however, I do not think that suddenly all across the world everybody's technique went boom! Something else happened, and although nobody has real evidence that there is more virulence in the 80/81 strain and none of us can prove it, I submit that some of us have suspicion that this might be the case. Now we talk about debilitated surgical patients being the ones that are infected, and they are, and the average age of our patients is older, and we are doing longer and harder operations, but we are seeing some vicious staphylococcal infections in young people that are having routine surgery, and we just wonder whether that might indicate that perhaps there is something we have not discovered yet about virulence or higher infectivity. Lastly, someone asked the question—do antibiotics, if given to a patient prophylactically, increase the chance of having an infection? Well, if you look at it in the case of the individual surgical patient receiving prophylaxis for a polybacterial flora, either in the genitourinary tract, the gastrointestinal tract, or respiratory tract, I think that the bulk of evidence indicates that what you would really do, or have the biggest chance of doing, is to change the flora from possibly sensitive strains to predominantly resistant ones. You have not done anything for or against setting up an infection but you have fixed it so that it is more likely that if infection develops it will be caused by one of these resistant strains. I think that if more studies like that of Petersdorf and Bennett on unconscious patients come along, where secondary infections developed—and these were skin infections—if you recall that article—only in the patients that had antibiotic prophylaxis it begins to appear that there is a little evidence that says, “Yes, you can make an individual patient more susceptible.” I do not know the answer, but these aspects certainly make me keep an open mind.

**Dr. Wise (Moderator):** Dr. Learner, what has been the results of the control program at Temple University?

**Dr. Learner:** We had about a 5 per cent infection rate of clean surgical wounds before we instituted control measures. After we started a program of control, the infection rate decreased to approximately 1 per cent.

**Dr. Wise (Moderator):** Dr. Howe.

**Dr. Howe:** We have been keeping track of our infection rate since 1949. We have a system of doing it, which we think is fairly accurate, but we may miss some, especially among the private patients. If it is not correct, it is on the low side rather than on the high side. We started with less than 1 per cent in 1949 and we reached our peak in 1953, when our over-all infection rate in minor and major wounds was around 4 per cent. These were clean wound cases only. We started a rather intensive program at that time, with carrier rate studies and so forth, and our infection rate came down the next two years to, about, 2 per cent. We said, “Look what we have done. The result of our efforts has caused this infection rate to fall.” We continued with the same program, and in 1956 another peak came up, just as high as the 1953 peak, and our carrier rate continued downward, and it was 43 per cent last year. Now I am not so sure that we did anything, and I am not so sure but that this thing might go in a natural fluctuating cycle. When you start a preventive program you must beware of attributing any fall in infection rate to what you have done until you see what the pattern is over the years. Like any infectious disease pattern, I think it probably has cyclic variations. Dr. Altmeier in Cincinnati has a sensitivity data curve for increased peaks of resistance to penicillin of a large number of strains of staphylococci which you can practically superimpose on our curve for infection rate data. Thus we must be careful not to attribute too quickly any fall in infection rate to some program that we have instituted last month or six months ago.

**Dr. Shaffer:** May I comment for a moment on that, Dr. Wise, because there are several factors that enter into this. Over a seven month period in which we studied the colonization of babies in the hospital and compared it with the carriers among the nurses, we found two things that were very clear. First that certain strains of staphylococci are more easily implanted than others, and that the carrier of one strain would very soon cause 100 per cent colonization; secondly that given two nurses in two separate nurseries, each of them carrying the same strain, there was always a variation in the ability of one or the other nurse to transfer her prevalent strain to the baby she cared for. Thus you might very well reduce your carrier rate down to 10 per cent and still have a higher infection rate if you had a good colonizer and a good disperser. If we just knew how to tell the nurse who was not a good disperser, it would be helpful. Recently, we took some of our carriers and put a gown on them and had them read from a textbook for 10 minutes and then put a vacuum cleaner on their gown to see whether they were transferring the staphylococci down onto their clothing. Unfortunately they all do this.

**Dr. Rountree:** I would like to make a comment, too, about some of these points. I think we must be careful about this. We have examples where one nurse only has been responsible for large epidemics, or one epidemic of infection in a nursery. Certain strains do apparently colonize more readily than others. That is something that we must remember. I think that it might be worthwhile drawing your attention to work that is being done at St. Bartholomew's Hospital in London, where studies have been made of endeavors to reduce the surgical wound sepsis rate. Like Dr. Howe, a number of measures have been taken in which reduction of sepsis rate resulted. The strains that were responsible for the wound infection were known; they have been typed and workers could see various phases in their experience. First, when they had bad theater ventilation and bad techniques they got infections with many types of staphylococci. Then they cured that, they fixed everything up, and they thought they were all right. Then they got a phase when one surgeon on the team became a source of infection. This was an epidemic with one type. That was disposed of, and then they instituted isolation proceedings because then they found they were getting a phase when patients were being infected in the wards, taking their staphylococci to the theaters with them and bringing them back again, after the patients had been isolated. All they have now are a very small number of wound infections that can be shown to be due, many of them, to penicillin-sensitive staphylococci of very many different types. These have come into the hospital with the patient, so that any evaluation of the efficacy of measures to control surgical infection rates, I think, must be at least accompanied by an appreciation of the different types of organisms that may be involved.

**Dr. Wise (Moderator):** Dr. Meleney.

**Dr. Meleney:** In the old days, about a third of surgical cases were problems of infection. There is no doubt that the antibiotics greatly reduced the number of surgical infections on the ward. A study that we began in 1925, when the percentage of wound infections in clean cases was 15 per cent, by closing all the doors, by which contamination entered, it was brought down to less than 2 per cent, which we thought was the irreducible minimum. Now it seems to be going up again. I do not think there is any question that the indiscriminate use of antibiotics has caused a letdown in sterile techniques. Confidence in the antibiotics also has caused a letdown in surgical techniques all over the country, and that has resulted in a rise in the incidence of infection in the last few years. The only way that it can be brought down is by further careful screening and study in any hospital that is serious about this matter. Now in regard to the matter of carriers, it seems to me that no one has talked very much about how to reduce the distribution of the organism by carriers. I would like to ask the panel what they think could be done in this regard and whether it would not be worthwhile to set up a half a dozen studies correlated with one another to investigate this problem of the distribution of the organism by

carriers. The only way to determine carriers is by taking cultures; the only way to determine whether they have got these hot strains is to use the phage screening technique. That takes time, and effort, and money, but that could be done in a half a dozen units. I believe that the local application of certain bactericidal antibiotics to the nasopharynx could reduce the distribution of the organism by the carriers. The recognition that a person is a carrier of itself would limit the distribution; he would wash his hands more frequently, he would avoid contact with the patients, he would be careful to use paper handkerchiefs instead of his own. If he knew he was a menace, that alone would reduce the distribution; but there could be further methods to reduce this distribution by the carriers, and I would like to see a half a dozen units set up for that purpose.

**Dr. Rountree:** We have done, not only trials, but we now have studied the use of nasal salves containing mixtures of antibiotics that are never used, or very rarely used. We have used mixtures of neomycin and bacitracin in creams, and we have typed or evaluated what success we have had with these methods. I would say from our experience that this treatment will remove staphylococci from the anterior nares of approximately 80 per cent of people if carried out conscientiously. We found that some people could not be cured. There are others in whom recolonization with the same strain occurs, and it seems clear in such cases these people have contaminated their environment, and the organisms have then been picked up again from their own bed clothing and their clothing. We find that if you want to eliminate nasal carriage, you must also stress the importance of the immediate and personal environment of the person with whom you are dealing. Now, I know that not many people in this country have used this method or advocated it but we found it to be valuable and it is practical up to a certain point. We think you should use two antibiotics to eliminate the possibility of a double resistant mutant. This would be a very very rare occurrence but we think you should use a substance that is not used for general therapy. Other people in England are using hibitane cream, and they are finding much the same sort of thing. What you are doing in effect is putting a chemical barrier between the *Staphylococcus* in your carriers and your susceptible population.

**Dr. Wise (Moderator):** Dr. Shaffer.

**Dr. Shaffer:** I would just like to comment about this same problem. For the past year we have been carrying out a study of the effect on the carrier state of nasal sprays containing antibiotics and hyaluronadase. We hope that before long we will have some definite information; but there seems to be some hope that at least you might be able to control the carriers while they are using the spray or the cream and let them continue their work. This is of practical importance to us, of course, with medical students and student nurses, who develop a carrier state and then have obligations that they must fulfill to graduate. We have tried the specific bacteriophage as a spray without any encouragement, but without any ill effects either.

**Dr. Learner:** Dr. Rountree, you mentioned that 80 per cent of the nasal carriers also had skin infections at one time or another. Have these people who have used the ointments also been free of the skin infections after the use of the ointment?

**Dr. Rountree:** That is a good point, too. We had to approach the problem of the person with the skin lesion, and what to do with people who are having recurrent chronic furunculosis. We stressed that these people are nearly always skin and nasal carriers of the infecting organism. These are the patients with the recurrent lesions. Antibiotic therapy is not needed with these people. One should try to cure their carrier state. If you can eliminate the *Staphylococcus* from their skin, then you can cure them. These people are treated with nasal ointments and also with bactericidal soap, used for washing. We have had considerable success in dealing with these people in this way.

**Dr. Wise (Moderator):** Dr. Rountree, Dr. Frank Sweeney and I have been interested in this same problem in the last two years. We have seen a number of people from homes in which there are small children who have developed abscesses. These furuncles have been recurrent for as long as two years. Some of these people have recorded as many as 200 boils, and in their family there may be several. We have also approached this with the thought that a reduction of the inoculum on the skin may reduce the number to a quantity less than necessary to produce an abscess. If this is true, and one can reduce the inoculum in a person with recurrent chronic furunculosis by removal of staphylococci, one should be able to cure the disease.

We have been having these people go through very rigid programs after they thoroughly understand the disease that they have, that they are infecting themselves by transfer from pus site to new site, and from pus to clothes, to new site, or from pus to bed linens and pajamas to new sites. Many of these people have had vaccination therapy, toxoid injections, and they have had antibiotics for as long as a year without relief prior to hygienic therapy. We have had them cover their mattresses and pillows with plastic covers. In the morning when they get up they have to take off their pajamas and their linens, which are laundered. They disinfect their mattress and plastic pillow covers, then they take a bath with soap and when they have finished this they clean around the abscess for at least 5 minutes with alcohol. Then they put a barrier of an antibiotic cream over the draining abscess. They do this four to six times a day, in order to keep their skin so clean that they cannot reinfect. We have also used antibiotics in the nose. We have had failures, but we have also had a great number of successes with this treatment. I have been impressed with the rigid use of good hygienic principles in the correction of this vicious cycle of recurrent furunculosis. We have not used antibiotics systemically in most of these cases.

**Dr. Rountree:** Well, I think that our experience completely parallels yours and we have certainly seen people who have had continued lesions for quite a number of years whose infections have been arrested when they finally adopted the kinds of measures that you have outlined.

**Dr. Wise (Moderator):** Are there any questions from the audience? Dr. Romansky.

**Dr. Romansky:** There is one area here that has not even been explored. I do not think there is an answer to it at present, but certainly it should be discussed. Dr. Howe talked about the question of increasing virulence, but no one has really touched upon it. I think the basic issue is, what has happened to the immunity factor in people since the time of the antibiotics? Dr. Shaffer mentioned that the internists are not seeing these infections; well, he ought to make a survey, I think, of his internists, because we are all seeing the same problem. I do think this is a question that needs great exploration—the whole factor of immunity, immunological techniques, methods of evaluating what there is in these people that makes them so susceptible to the *Staphylococcus*. Maybe Dr. Rountree has something on this, but this is an area I think that we must explore. I disagree with Dr. Knight that these infections are occurring primarily in the debilitated and those with serious underlying diseases. We are seeing them in people of all ages in apparent good health.

**Dr. Rountree:** Yes, that might be true, but newborn babies have been the same for a long time.

**Dr. Knight:** But they just started leaving the vernix on about 10 years ago.

**Dr. Krantz:** My name is Laage Krantz, from Stockholm. I would like to ask the panel if there is any place for the use of ultraviolet irradiation for preventing spread of staphy-

lococcal infections? There are many people in this country who are interested in this problem, too. For instance, Dr. Stokes, in Philadelphia, who definitely thinks that if you use ultraviolet sterile lamps in your nurseries and wards that it would be of some help.

**Dr. Wise (Moderator):** Dr. Howe, would you like to discuss the use of irradiation in the operating room?

**Dr. Howe:** Well, this problem is being studied now by the National Research Council. There have been several units set up. It was discussed at a recent meeting of the National Research Council and there were some proponents who believe that it was successful. However, in my opinion, because of the inaccuracy with which the infection rate had been checked it was not possible to come to any conclusion over the years as to whether the ultraviolet was really effective. Some of the objections to it are: the difficulty in using it, the screening that you have to use for personnel, which makes it impractical to use, and be acceptable, in all hospitals; the screening effect of dust particles; and the hazards to skin of burning. I do not think we have an answer to it but I think that in two or three years we will have some good information on it through the studies that are now just starting.

**Dr. Wise (Moderator):** Our time is almost up. Dr. Finland, you had your hand up a little earlier. Do you still have a question you would like to ask or a comment you would like to make?

**Dr. Finland:** I want to make two comments. One is this, that if *Staphylococcus* causes infection, then it may produce epidemics, and we do not understand some of the basis of epidemics of staphylococcal infections. Moreover, this is not the first time we have had epidemics. As Dr. Shaffer mentioned, it goes far back. For example, in 1918 during the war there were areas in which 50 per cent of the deaths in Army camps were due to staphylococcal pneumonia. In 1941 we had in the United States an epidemic of staphylococcal infections; in 1953 we had a similar epidemic of staphylococcal infection in many areas, and now we are having a resurgence of it. There is something that carries it. It occurs simultaneously with infections with the influenza virus, and whether that has some kind of an effect either on the population or on the organism I do not know, but we know of many virus infections that have been discovered—some of them may be predisposing to staphylococci. The other point that I wanted to make is that there is some reason to believe that antibiotic therapy does predispose to infection because infection requires not only an organism but it requires a certain inoculum; and also, in the host, there is some interplay between the components of the flora. For example, when we were giving oxytetracycline to patients with various diseases, we had an epidemic of staphylococcal diarrhea in which there were pure cultures of staphylococci. That was a result of the combination of two factors, one specific and the other an alteration of the bowel flora. This may be true whenever we give patients antibiotics, we remove certain elements of their flora, which permit other elements, and it may be the *Staphylococcus*, to multiply to a point where you have an infectious inoculum.

**Dr. Wise (Moderator):** Thank you, Dr. Finland. Our time is drawing to a close. This is indeed a complicated subject; there are many things that we could discuss, such as host factors, treatment of the patient to improve staphylococcal infection, factors of immunity, the importance of antibiotics in the selection of these resistant strains, and many others. I think we will conclude the discussion at this point. I want to thank all members of the panel for their excellent discussion and those in the audience who have participated.



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